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CLINICAL ANALYSES OF FACTORS INFLUENCING L DOPA TREATMENT OF PARKINSON'S SYNDROME

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Abstract. One hundred and twenty-three patients with Parkinson's disease have been treated with L-dopa for 6–31 months. Rigidity was improved in 85%, hypokinesia in 93%, tremor in 69%, speech disturbances in 30% and mental depression in 48% of the patients. The existence of any correlations between measured variables was tested in 260 tests. The L-dopa dosage was higher in men than in women. Improvement in ADL disability was inversely correlated to age. This improvement was directly correlated to the occurrence of tremor before treatment and to the appearance of involuntary movements during treatment. There was correlation between the appearance of involuntary movements and the appearance of muscular hypotonia. These and other observations allowed conclusions concerning the proper indications for L-dopa treatment of Parkinson's syndrome.

When this study was started in May 1968, oral L-dopa treatment had been used only in a few clinical trials (5, 8, 13, 15). In the initial study the necessity for objective methods to evaluate the possible effects of L-dopa determined the design of the investigation (2). The beneficial effects on Parkinson's syndrome soon became obvious, as well as the risks of side-effects. At this early stage of the L-dopa treatment patients with severe functional disabilities were selected. The indications for treatment were obvious even when the risk of side-effects was considered. Because of the expected influence of L-dopa treatment on the blood pressure, advanced cardiovascular and cerebrovascular disease was considered a contraindication.

In the further studies on a larger patient material presented here our interest was focused on the proper indications for L-dopa treatment and the diagnostic criteria of Parkinson's syndrome. As mental side-effects were evident in our pilot study (2), attempts were made to investigate the influence of L-dopa on mental functions. The present report concerns a follow-up of 134 patients with Parkinson's syndrome. The patient material includes both a younger out-patient clientele in a neurological department with Parkinson's syndrome as a single disease, and elderly patients in a geriatric department, many of whom having other concomitant diseases.

SUBJECTS

The material includes 134 patients, 68 women and 66 men, in whom L-dopa treatment had been instituted before July 1, 1970. The follow-up was performed in Dec. 1970. At that time 123 patients, 66 women and 57 men, had been treated with L-dopa for 6 months or more. Most of the patients treated are living in Göteborg, while some were sent to us from other parts of Sweden. The mean age at the appearance of the first symptoms, later on diagnosed as Parkinson's syndrome was 55±9.9 years ($M \pm$ S.D.). The mean age of the patients at the start of the treatment was 63±8.9 years ($M \pm$ S.D.). The duration of the disease at the start of the treatment was 8±5.4 years ($M \pm$ S.D.). In 13 cases Parkinson's disease was known to have occurred in parents or siblings. Twenty-three patients had had encephalitis, 11 of them during the period 1918–21. Stereotactic operations had been performed unilaterally in 16 and bilaterally in 11 cases. Fig. 1 shows the age distribution at the beginning of the disease and at the start of the treatment. Fig. 2 illustrates the duration of the disease when L-dopa treatment was started. At the follow-up the duration of treatment varied between 6 and 31 months in the patients still on L-dopa therapy ($n = 118$), as illustrated in Fig. 3. A further five patients had been treated with L-dopa for 6 months or more, but the treatment had been withdrawn in four, one of whom died after the withdrawal, and one died while on L-dopa just at the time of this follow-up. Thus the total group treated for 6 months or more numbered 123 patients. In 11 cases, in whom L-dopa treatment had been withdrawn because of side-effects, lack of cooperation or other diseases than Parkinson's syndrome, the duration of the treatment had varied between 0 and 3 months.

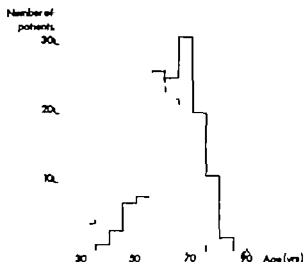


Fig. 1 Age at beginning of parkinsonian symptoms ( ) and at start of L-dopa treatment (—).

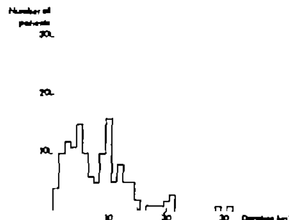


Fig. 2 Duration of parkinsonian symptoms at start of L-dopa treatment. Since many patients could not exactly remember this duration, peak values are seen at 5 and 10 years of duration.

METHODS

During the first 6 months a detailed single-blind study was performed in 6 of the patients (2). The further studies followed the same schedule of oral L-dopa administration. The initial dose was 200-250 mg L-dopa 3 times a day together with food. The dose was then successively increased by 400 or 500 mg a week until a sufficient reduction of the parkinsonian symptoms was reached or dose-limiting side-effects appeared. During the later part of the study (i.e. since Jan. 1970, the treatment with L-dopa was usually initiated in ambulatory practice.

Many of the patients had received physical therapy aimed at counteracting rigidity, muscular atrophy and rigidity continuously or periodically before L-dopa treatment was started. During the present study a training program was used both for in- and out-patients.

During the first 6 months of the study a number of objective tests were performed in order to evaluate the effect of the treatment. These included writing and drawing tests, measurement of time needed to walk 10 m, to rise from and return to sitting position, and to put on a pair of socks. They further included cinematography and mechanographic examinations and the scoring of nursing load and motor impairment (?).

Later on simplified evaluation methods were used. Thus the cardinal symptoms hypokinesia, rigidity and tremor, as well as speech disturbances, were graded as absent (0 score), moderate (1 score) or severe (? scores). Hypokinesia was considered severe when voluntary hand or walking movements were impaired. When hypokinesia influenced mainly associated and synergic movements, the inability was considered moderate. Rigidity was considered severe when a continuous muscular resistance during passive extension in elbow, wrist or knee joints had to be overcome by a certain resistance. When this muscular resistance was clearly recognized but nevertheless the joint could still be bent easily the rigidity was considered moderate. Tremor was evaluated as severe when it obviously interfered with voluntary motor actions, and as

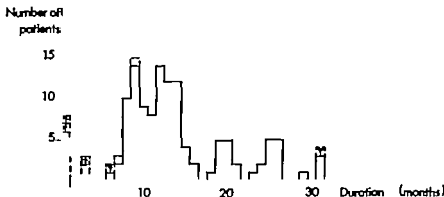


Fig. 3 Duration of L-dopa treatment at follow-up. □ = patients still on L-dopa (= 118), □ = patients, deceased or no longer on L-dopa (= 16).

moderate when it was less pronounced and did not interfere markedly with the ADL functions. The dysarthria or aphonia were considered severe when they reduced the patient's ability to make himself understood and as moderate when, although the voice was low and hoarse, the patient could easily make himself understood.

The general motor and ADL functions were scored according to the following scale

0. The patient was free from parkinsonian symptoms during most of the day
1. The parkinsonian symptoms were clearly recognizable but did not interfere markedly with the ADL functions.
2. The parkinsonian symptoms interfered with the ADL functions but the patient did not require care.
3. The parkinsonian symptoms interfered markedly with the ADL functions and the patient needed some care because of the disease.
4. The parkinsonian symptoms made the patient completely disabled as far as ADL functions were concerned.

Changes in the parkinsonian symptoms which moved the patient from one of these functional disability groups to another were considered significant.

As some patients in the pilot study revealed marked mental changes during L-dopa treatment (?), the thorough penetration of the case history included a detailed psychiatric anamnesis and careful, repeated examination of the mental state.

The general clinical examination included ECG and chest X-ray and in the majority of cases EEG before and on certain occasions also during the treatment. BP was followed regularly in both erect and supine positions.

The following laboratory tests were performed at regular intervals during the treatment: Hb, WBC with differential count, thrombocytes, total carbon dioxide, fasting blood sugar ESR on whole blood, PBI, creatinine, bilirubin, alkaline phosphatase, thymol, transaminases, sodium, potassium, calcium, chlorides, phosphate, and protein on serum.

The series of patients was characterized as regards age distribution, duration of disease, symptomatic picture duration of treatment, doses of L-dopa, and earlier operative treatment, as well as the effect of the L-dopa treatment on the parkinsonian symptoms, and the appearance of side-effects. Possible correlations between these characteristics were searched for

To test the existence of any correlations between measured variables, the χ^2-test for trend in contingency tables was used (17). When many tests are performed simultaneously as in this investigation, it is important to have a low level of significance in the single test in order to not get too many false significances. The expected number of false significances is not greater than αN here α = the level of significance and N = the number of tests. In this study we have chosen $\alpha = 0.01$ and have performed $N = 260$ tests. This means that the expected number of false significances is less than 3. The calculations were made on IBM 340/65 at the University of Göteborg Computing Center.

RESULTS

When this follow-up was performed, 118 patients, 62 women and 56 men, out of 134 patients in whom L-dopa treatment had been initiated, were still on this treatment. All except one showed some improvement, and the treatment of this single patient was withdrawn soon after the follow-up.

Two patients had died while on L-dopa treatment, one from bronchial carcinomatosis and the other from unknown cause. The latter in whom L-dopa treatment was known to have produced postural hypotension was found dead in his home. Autopsy was not performed. One patient developed bilateral pneumonia with septicemia after 1 month of treatment, and L-dopa was then withdrawn. He died 1 week later. In one patient no effect of the parkinsonian symptoms was seen and when after 6 weeks of treatment, he suddenly became stuporous, the therapy was withdrawn ( ). His mental condition improved only very little after this and he died a year later in pyelonephritis with septicemia. In two cases postural hypotension appeared which caused withdrawal of the L-dopa therapy as one of them had coronary insufficiency and the other congestive heart failure. The latter died months later because of the heart disease. One patient got a myocardial infarction while on L-dopa which consequently was withdrawn. In four patients hallucinations or mental confusion, not previously recognized, appeared during the treatment, which therefore was withdrawn. In one patient nausea made further treatment impossible. In four cases the treatment was withdrawn because of lack of cooperation of the patient.

Except for the 118 patients still on L-dopa a further five (four women and one man) had been treated for 6 months or longer viz. the patient who died of bronchial carcinomatosis, the patient who died of congestive heart failure, the patient with a myocardial infarction, and two of the patients in whom treatment had been withdrawn because of lack of cooperation. The remaining 11 patients had been treated between 0 and 3 months. The present report will be restricted to these 118 + 5 thus 123 patients in all as far as the therapeutic results are concerned.

The duration of treatment with L-dopa was 14.2 ± 6.3 months ($M \pm S.D.$) (Fig. 3). The first optimal dose (Fig. 4) was on an average 3.8 ±

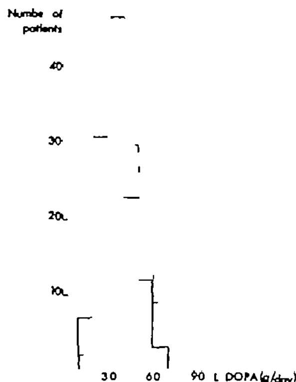


Fig 4 First ( ) and second (—) optimal L-dopa doses (= 123).

1.2 g/day in the female patients 3.5 ± 1 1 g/day and in the male patients 4.. ± 1.2 g/day The first timal dose was reached already within 1 month 19% after 2 months by 36% 3 months by % 4 months by 9% and after 5 months by % of the patients. In 59 cases the optimal dose at the follow-up was lower than the optimal dose during the initial period in 27 of them the dose had been lowered by 1 g or more. In ten cases the optimal daily dose had been increased, in four patients by 1 g or more. In the other 54 patients the dose which during the initial treatment period was found to give the maximal response was still the optimal one (Table I). At the follow-up the average L-dopa dose was 3 4 ± 1 1 g/day for women 3 1 ± 1 0 g/day and for men 3 8 ± 1 1 g/day Body weight was not determined in all patients, since some of them were initially bedridden and so severely handicapped that the ordinary scales could not be used. Therefore the material could not be evaluated as to a possible correlation between b.wt and dosage.

The first positive effect of the treatment was usually a reduction of hypokinesia followed by a decreased rigidity Subjective effects were often reported already after 2–3 weeks of treatment but objective effects were usually not reached until after 1 month, in a few cases not until after 3–5 months of treatment. An obvious reduction of the tremor was usually not observed until several weeks or months, or in some cases more than 1 year after the effect on hypokinesia and rigidity had been observed. The initial therapeutic effect, which usually reached a maximum after 2–4 months, was in most cases followed by slower but progressive improvements of the general condition which seemed to be related mainly to the increased physical and mental activity secondary to the general improvement.

According to the scores the rigidity was reduced from 1 6 ± 0 04 to 0 6 ± 0 05 the hypokinesia from 1 7 ± 0 04 to 0.6 ± 0 05 and the tremor from 1 4 ± 0.06 to 0.6 ± 0 06 (M ± S.E.) The distribution of the scores is illustrated in Fig. 5 The rigidity was improved in 85% the hypokinesia in 93% and the tremor in 69% of the patients Speech disturbance was present in 88 patients before treatment. Speech improved obviously during treatment in 30 patients.

The average value of ADL disability (5-grade scale) was 3 0 ± 0.07 and decreased during treatment to 1 6 ± 0 10 (M ± S.E.). The distribution is illustrated in Fig. 5 Among the patients treated for 6 months or more the improvement expressed in number of grades (Table II) was 4 3 2, 1 and 0 grades in 4 11 30 64 and 14 patients, respectively In 13 of these last 14 patients the treatment caused some improvement as judged by a lower degree of rigidity hypokinesia or tremor Thus only one of these patients did not show any objective sign of physical improvement.

Sixty-one of the patients who before treatment belonged to ADL groups 3 and 4 i.e. were dependent on care improved to ADL groups 0, 1 or 2, i.e were no longer dependent on care

Mental depression was common. In 10 patients

Table I. *Changes of L-dopa doses (n = 123)*

No. of pats.

With unchanged dose	With increased dose		With lowered dose	
	>1 g	<1 g	>1 g	<1 g
54	4	6	27	32

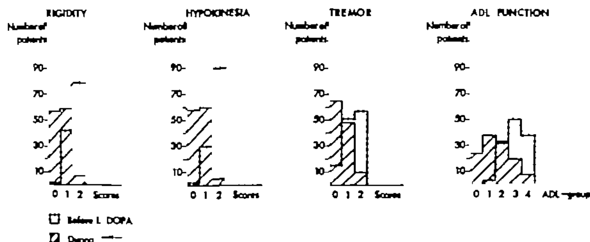


Fig. 5. Changes in rigidity, hypokinesia, tremor and ADL function ($n = 123$).

with mental depression at the start of the L-dopa treatment the depression disappeared, and in a further 10 the symptoms were markedly reduced during the L-dopa treatment. Twenty-two patients had depressive symptoms which did not improve during the L-dopa treatment.

Twenty-seven of the patients had earlier been treated with stereotactic operation. A comparison of the effect of L-dopa on these patients and on the total patient material showed that 8 versus 15 improved 3 or 4 ADL scores, 5 versus 30 improved 2 scores, 8 versus 64 improved 1 score and 4 versus 14 did not change ADL score. Thus the therapeutic results were not obviously influenced by previous stereotactic operation.

Side-effects (Table III)

Vegetative side-effects. Nausea, usually without vomiting, was the most common side-effect appearing in 50% of the patients either during the initial period, continuously during the whole period of treatment, or at high doses. When nausea appeared during the initial phase, it usually started shortly after the morning dose, lasted a few hours and was dependent both on the size of the dose and on the rate of the dose increase. Even at a rate of dose increase as slow as in the present study half of the material complained of nausea. The nausea usually disappeared spontaneously within a few weeks of treatment. In one patient nausea persisted and made further treatment impossible in spite of several attempts to administer L-dopa in frequent smaller doses together with food. Furthermore, several patients complained of lowered appetite and slight nausea continuously during L-dopa treatment. In a few cases, 11 out of 123, nausea was markedly exaggerated at high dose levels and thus limited further increase of dose. For example, one woman tolerated 2.8 g/day well but without optimal effect on the parkinsonian symptoms. Repeated attempts to increase the dose to 3 g/day, which further improved the parkinsonian symptoms, invariably produced nausea.

The L-dopa treatment caused postural or orthostatic hypotension in 21 patients. The changes were considered significant if the systolic BP in supine position changed more than 20 mm, or the diastolic BP more than 10 mm, when the patient changed to erect position. Hypotension necessitated lowering of the L-dopa dose in two and withdrawal in two cases.

In two patients hypertension appeared during treatment and disappeared when the L-dopa dose was reduced.

In a 55-year-old male with obvious parkinsonian symptoms since 1959 repeated controls of the BP had been performed for 5 years showing values 130–150/90 mmHg.

Table II *Change of ADL group during L-dopa treatment* ($n = 123$)

Before L-dopa		During L-dopa	
ADL group	No. of pats.	ADL group	No. of pats.
4	39	4	8
		3	17
		2	6
		1	4
		0	4
3	50	3	3
		2	25
		1	15
		0	7
2	31	2	2
		1	18
		0	11
1	3	1	1
		0	2

Table III. *Side-effects appearing during L-dopa treatment*

Symptoms	No. of pats.	Causing: Lowering of L-dopa dose	Causing: Withdrawal of L-dopa
Nausea	67	11	1
Hyperkinesia	66	22	
Muscular hypotonia	24	6	
Mental confusion	10	6	4
Mental depression		2	
Mania, hypomania	5	1	(1)[a]
Arterial hypotension	21	2	2
Arterial hypertension	2	2	

[a] This patient also had a mental confusion which was the dominating symptom.

In Jan. 1970 the BP was 150/90, ECG and heart volume were normal and no signs of retinopathy or nephropathy were present. Heart frequency at rest was about 70 beats/min. Treatment with L-dopa started on Feb. 5 1970. On March 18, when the L-dopa dose was 5.75 g/day the BP at rest had increased to 200/140 mmHg and the heart frequency was 108/min. During the following weeks the L-dopa dose was lowered to 3.75 g/day. On April 8 the BP was 160/120 mmHg and the heart frequency 100 beats/min. The dose was then lowered to 3 g/day. The BP on May 6 was 150/115 mmHg and the heart frequency 96 beats/min. On June 19 the BP was 140/95 mmHg, and on several occasions thereafter about 135/90 mmHg, and the pulse frequency about 70 beats/min.

In another patient with earlier known hypertension treated with hypotensive drugs, the addition of L-dopa in doses of more than 5 g/day increased the BP while lower L-dopa doses had no obvious influence.

In six cases post-menopausal vaginal bleedings appeared. Two women were 49 and 51 years old, the age of the remaining cases ranged between 56 and 62 years. In seven cases (3 women and 4 men) increased libido was reported. Two of them were more than 75 years old. However, this effect was not systematically asked for.

Mental side-effects. The most common mental change, correlated in time to the L-dopa treatment, was confusion, which appeared in 10 of the 134 patients. In six of these cases the symptoms disappeared when the L-dopa dose was lowered and in the remaining four when the L-dopa treatment was withdrawn. In a further 12 cases mental confusion had appeared on several occasions previously and was not obviously influenced by varying doses of L-dopa. Most of these patients were, however, also on anticholinergic therapy but, on lowering of these doses, no influence on the mental symptoms was observed.

Five patients showed obvious hypomanic or manic symptoms. In two of them the symptomatology also included confusion. In all of them the symptoms developed during a rather high L-dopa dose. In one of the cases with manic confusion the L-dopa had to be withdrawn, in the other the symptoms seemed to be dose-dependent, as he was able to continue the treatment on a lower dose. In the remaining three cases it was not necessary to change the dose, as the hypomania was tolerable. Two patients suffered from mental depression, not earlier recognized. When the L-dopa dose was lowered and imipramine added, the symptoms disappeared. A further four patients with a history of depression developed depressive symptoms during the L-dopa therapy. In these cases we chose to keep the same dose of L-dopa and, as above, to add imipramine, with good effect.

Neurological side-effects. The most frequent neurological side-effect was hyperkinesia, occurring in 66 patients, i.e. in around 50% of the material. The hyperkinesia was dependent on dose level, with great individual variation. Thus involuntary movements in some cases appeared at doses below 2 g/day and in others at 7–8 g/day. Furthermore, the dose level provoking hyperkinesia decreased in many cases during prolonged treatment.

In some cases no dose level could be found at which the patients were free from parkinsonian symptoms and involuntary movements. The patients had to accept a dose high enough to produce hyperkinesia in order to eliminate or diminish parkinsonian symptoms. The involuntary movements appeared in various muscle groups. Tongue and mouth movements were common. The extremities, too, often showed involuntary movements, whereas trunk hyperkinesia was seen in a few cases only. In hemiparkinsonism hyperkinesia in some cases was seen in the intact and in other cases in the affected half of the body. In patients with previous thalamotomy the hyperkinesia more often appeared ipsilaterally but in a few cases also contralaterally. The character of the hyperkinetic movements was extremely variable. Choreatic, athetotic and myoclonic movements occurred. In some patients the hyperkinesia was continuous during the daytime, but more often it appeared during a certain period after L-dopa dose.

In 24 cases the initial muscular hypertonia was replaced by hypotonia. In some cases this was revealed as an intensive fatigue, particularly in the legs, but in some cases the muscular hypotonia appeared so suddenly that the patient might fall to the floor. Six patients had traumatic orthopedic complications such as bone fractures. Four of these had a marked hypotonia, and a correlation between muscular hypotonia and the accident was suspected.

Peripheral paresis of nervus radialis appeared in five patients during L-dopa treatment.

Statistical correlation analysis

In the statistical analysis of the material the following observations were used:

Sex, heredity for parkinsonian symptoms, encephalitis, thalamotomia (which side and when it was performed), age at onset of parkinsonian symptoms, duration of parkinsonian symptoms, age at start of L-dopa treatment, duration of L-dopa treatment, first optimal L-dopa dose and when this was reached, second optimal L-dopa dose and when this was reached, reason for changing dose, reason for withdrawal of L-dopa, rigidity before and during L-dopa treatment, hypokinesia before and during L-dopa treatment, tremor before and during L-dopa treatment, speech disturbances before and during L-dopa treat-

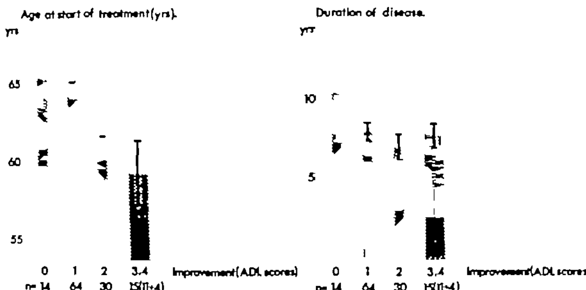


Fig. 6. Relation between age at start of L-dopa treatment and ADL improvement and between duration of disease and ADL improvement ($n = 123$).

ment, ADL function before and during L-dopa treatment, improvement in ADL disability EEG before and during L-dopa treatment, hyperkinesia during L-dopa treatment, muscular hypotonia during L-dopa treatment, mental depression before and during L-dopa treatment, mental confusion before and during L-dopa treatment, hypomania or mania before and during treatment, nausea during L-dopa treatment, history of gastritis of ulcus duod. a. ventr. arterial hypotension before and during L-dopa treatment.

Of the 260 tests performed, 45 showed significant results on the 0.01 level. This means that most of the significances can be expected to be results of true correlations, as the expected number of false significances is less than 3. Of the significant results obtained we will mention the following:

1. Improvement in ADL disability (number of scores) was negatively correlated to age at the start of L-dopa treatment (Fig. 6). The effect of L-dopa on the physical ability of the patients was thus better in younger than in elderly individuals.

2. Improvement in ADL disability was positively correlated to the occurrence of tremor before treatment. Patients with tremor showed greater improvement in ADL function than those without tremor. A good effect on ADL function was also positively correlated to a good effect on tremor.

3. Improvement of tremor was negatively correlated to the duration of the disease.

4. Improvement of speech disturbances was negatively correlated to age.

5. Improvement in ADL disability was positively correlated to the appearance of involuntary movements during treatment (Fig. 7).

6. The appearance of involuntary movements was positively correlated to the duration of treatment.

7. The appearance of involuntary movements was positively correlated to the appearance of muscular hypotonia.

8. The appearance of muscular hypotonia was positively correlated to the duration of treatment.

9. The appearance of muscular hypotonia was positively correlated to the appearance of nausea.

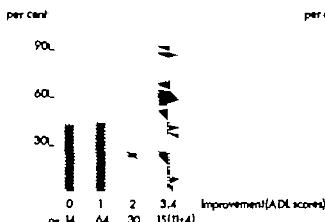
10. The appearance of muscular hypotonia was negatively correlated to age.

11. The appearance of mental confusion during treatment was positively correlated to age.

12. The average first optimal dose of L-dopa was lower in women than in men, and this difference was observed also for the optimal dose found later.

COMPARISON OF RESPONSE IN DIFFERENT HANDICAP GROUPS

The ADL grouping used implies that only patients with advanced handicap can improve maximally. Therefore, in each group of patients initially belonging to ADL groups 4, 3, 2 and 1 correlations were calculated for improvement in ADL disability to age (Fig. 8), for occurrence of tremor before treatment, for improvement of tremor during treatment, and for appearance of involuntary movements during treatment. These comparisons showed similar tendencies to those demonstrated by correlation analyses of the whole material, although the same degree of statistical significance was not found in these smaller patient groups. In ADL groups 4 and 2



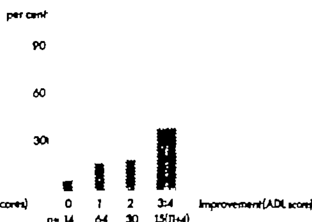


Fig 7 Relation between appearance of hyperkinesia and ADL improvement and between muscular hypotonia and ADL improvement ($n = 123$).

there was a probable negative correlation ($p <$ 0.05) between age and therapeutic effect. When dependence between the discontinuous variable (improvement in ADL disability) and the dichotomous variables (the occurrence of tremor before treatment, improvement of tremor during and involuntary movements during) was analyzed by the χ^2 test, a relation was observed only in ADL group 3 con- ing improvement of tremor during treatment ($p < 0.025$) and occurrence of involuntary movements during treatment ($p < 0.05$).

Laboratory findings

Changes in the hematological picture or in the renal function which could be related to L-dopa treatment were not observed. In some patients a positive ketostix reaction in the urine appeared, but these patients did not show any signs of ketonuria. It was found that positive ketostix reaction could also be produced by L-dopa. A darkening of the urine due to L-dopa oxidation was often observed, especially when an alkaline urine had been exposed to air and light. Hypopotassemia was observed in about 10% of the material. Ordinarily it could be substituted by peroral potassium. Further studies on this finding are now in progress.

DISCUSSION

It is remarkable that some patients with advanced parkinsonian symptoms were made totally symptom-free by L-dopa treatment, at least during considerable parts of the day and that there were very few patients in whom no effect at all on the parkinsonian symptoms could be reached. It is difficult, however to evaluate to what extent the physical training program caused some of the improvement. The observed improvement was correlated to a certain L-dopa dose level. The treatment can in certain cases eliminate the syndrom in toto. This strengthens the arguments in favour of the theory that the dopamine deficiency is a causative factor of all elements in the syndrome. Several earlier reports indicate that a lack of dopamine in substantia nigra and neostriatum and a defective transmission from nigrostriatal dopamine neurons cause Parkinson's syndrome (1 3 4 6, 7 9 10, 11 14 16). The clinical results of L-dopa treatment support the hypothesis that Parkinson's syndrome is not only a symptomatological entity but also a disease caused by a defined defect of the dopaminergic function.

The cause of the dopamine deficiency in the nigro-striatal dopamine neuron system does not seem to be a general lack of dopamine precursors in the body. This is rendered likely by recent studies (18 19) on the resorption and metabolism of phenylalanine showing that the plasma level and the rate of disappearance of this amino acid from plasma are the same in patients with Parkinson's disease and matched controls.

The essential pathogenic factor seems to be a

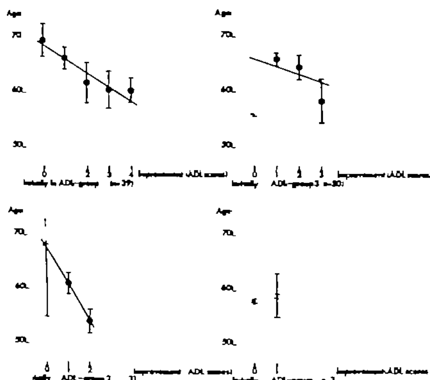


Fig. 2. Age in relation to improvement of ADL disability in different ADL groups. $M \pm S.E.$ and the regression line calculated on individual observations. ○ = the value of one patient or the mean value of two patients.
No. of pats. in each ADL group is given in Table II

defect in the rate of dopamine synthesis, in which the rate-limiting step is the L-tyrosine hydroxylation to L-dopa. L-dopa is then decarboxylated to dopamine. Dopamine does not penetrate the blood-brain barrier and can, therefore, not be used in the treatment of Parkinson's syndrome.

The dopamine deficiency in the substantia nigra and *neostriatum in Parkinson's disease may* however also (alternatively) be due to a partial or total lack of dopaminergic nigro-striatal neurons. If there is a complete degeneration of dopamine neurons, L-dopa administration cannot give rise to transmitter release from presynaptic dopamine terminals. If presynaptic dopamine neurons are degenerated, dopamine might be formed by decarboxylation of the administered L-dopa in other structures (other monoaminergic neurons—5 HT or NA—or capillary wall cells) and act on postsynaptic receptors made supersensitive by the degeneration of the presynaptic neurons. The latter theory has recently been strongly supported in an animal experimental work by Ungerstedt (21) who demonstrated a denervation supersensitivity in the postsynaptic receptors after degeneration of the nigro-striatal dopamine neuron system.

As long as the primary enzyme defect in loco cannot be corrected, L-dopa administration represents the closest approximation to a causal therapy. The present study, as well as earlier reports, *shows that an optimal L-dopa substitution* is generally not reached and that side-effects can usually not be avoided at L-dopa doses giving full restitution of the parkinsonian symptoms. It is conceivable that systemic L-dopa substitution therapy aimed at restoring normal dopamine transmission in certain dysfunctional nigro-striatal synapsis may result in effects on the transmission also in other catecholaminergic synapsis. Thus other dopamine and noradrenaline neurons in the central nervous system and peripheral noradrenaline systems are likely to be influenced by L-dopa administration.

The observation of a correlation of good therapeutic effect and the appearance of involuntary movements and/or muscular hypotonia

Table I *Patients with rheumatoid arthritis (age estimated tion some laboratory data and results of PAS staining immunofluorescence staining of skin biopsies)*

A = dermal-epidermal junction, B = sub-epidermal connective tissue, C = walls of small dermal vessels.
Grading of PAS staining: 1 = normal staining of dermo-epidermal zone, normal thickness of vessel walls, 2 = slightly increased staining of dermo-epidermal zone, slightly increased thickness of the vessel walls; 3 = heavily increased staining of the dermo-epidermal zone, heavy thickening of the vessel walls.
Grading of fluorescence staining: 1 = very slight fluorescence, 2 = distinct but not bright, 3 = bright fluorescence (See Material and methods).

Pat. no.	Age (y)	Duration (y)	SR (mm/h)	Electrophoresis Albumin (g/100)	γ-glob (ml)	RA factor titre	PAS A	PAS C	Albumin A	B	C	β_{1C}-glob. A	B	C	Fibrin A	B	C	IgG-glob. A	B	C	IgA-glob. A	B	C	IgM glob. A	B	C
Females (n 16)																										
5	59	14	106	2 3	2 8	320	1	1	1	2	1	1	1	1	1	1	2	2	2	2	1	1	1	1	1	1
7	50	8	23	3 5	1.6	80	1	1	1	2	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1
8	56	17	66	4 0	2 5	80	1	1	1	3	1	1	1	1	1	1	1	1	3	1	2	1	2	1	1	2
9	33	5	76	3 4	2 3	370	1	1	2	3	1	1	1	1	1	1	1	2		1	1	1	1	1	1	1
13	60	4	65	3 6	1 5	320	3	1	2	3	2	1	1	2	2	1	1	2	2	2	1	1	1	1	1	2
19	64	11	109	3 2	1 6	320	3	2	3	2	2	1	1	2	1	1	1	3	2	3	2	1	2	1	1	3
4	54	3	31	4 0	1.5	320	2	1	3	2	1	1	1	1	3	1	1	3	2		1	1	1	1	1	1
27	49	31	30	3 4	1.9	80	2	2	3	2	1	1	1	1	1	1	1	2	2	2	1	1	2	1	1	2
32	56	3	47	3 3	1 2		2	2	3	2	2	1	1	2		1	1	3	3	3	3	1	2	1	1	2
33	47	6	32	3 6	2.2	80	2	3	1	3	1	1	1	2	1	1	2	2	2	2	2	1	2	1	1	2
35	57	1	55	3 9	1 7	40	2	1	1	2	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1
36	39	5	6	3 8	1 6	20	2	2	2	2	2	1	1	1	1	1	1	2	2	3	1	1	1	1	1	2
37	54	8	61	3.3	1 6		2	1	3	2	1	1	1	1	3	1	1	3	2	3	2	1	2	1	1	2
42	53	3	10	4.5	1.0		2	1	3	2	2	1	1	2	1	1	1	2	2	2	1	1	1	1	1	1
43	59	12	50	3.5	1 7	160	1	2	1	3	1	1	2	1	1	1	2	3	3	3	1	2	1	1	1	2
47	65	13	70	3 4	2.1	640		2	1	2	1	1	1	1	1	1	1	2	3	2	1	1	1	1	1	2
Males (12)																										
1	51	13	33	4 4	1 7	640	1	1	1	3	1	1	1	1	1	1	1	2	3	2	2	2	1	1	1	1
10	61	9	124	2.7	2.8	320	2	1		3	1	1	2	1	1	1	1	2	2	1	2	2	1	1	2	1
11	64	22	27	4.3	1.5	160	1	2	2	3	2	1	1	2	1	1	2	3	3	3	1	2	3	1	1	2
28	63	3	40	4.1	1 9	40	1	1	3	3	1	1	1	1	1	1	1	2	3	2	1	2	2	1	1	1
29	63	5	118	3.1	2.3	640	2	2	3	3	2	1	1	1	3	1	1	3	2	2	2	1	1	1	1	1
31	57	3	13	4.1	1 7	80	1	1	3	2	2	1	1	2	1	1	1	3	3	2	1	1	1	1	1	1
34	55	1	15	4.1	1 8	80	2	2	2	2	1	1	1	1	1	1	1	2	2	2	1	1	1	1	1	1
38	56	1	34	3.2	1 4	80	3	2	3	3	1	1	1	2	1	1	3	3	3	3	1	1	2	1	1	2
39	58	16	115	4 1	1.9	80		1	3	2	1	1	1	2	1	1	1	3	2	2	2	1	1	1	1	2
40	26	2	79	4 2	1 6		1	1	2	2	2	1	1	2	1	1	1	3	2	3		1	2	1	1	3
43	42	18	16	4.3	1 7	20	2	1	1	3	1	1	1	3	2	1	1	2	3	3	2	1	3	1	1	2
46	52	24	32	3 1	3.3	80	2	1	2	2	1	1	2	3	1	1	3	3	3	3	3	2	1	1	1	1

Table II. *Patients without diagnosed rheumatoid arthritis (explanation, see Table I)*

Pat. no	Age (y.)	SR (mm/h)	Diagnosis	Immunofluorescence in skin tissue indicating presence of: PAS A	PAS C	Albumin A	Albumin B	Albumin C	β_{1C}-glob. A	β_{1C}-glob. B	β_{1C}-glob. C	Fibrin. A	Fibrin. B	Fibrin. C	IgG-glob. A	IgG-glob. B	IgG-glob. C	IgA-glob. A	IgA-glob. B	IgA-glob. C	IgM-glob A	IgM-glob B	IgM-glob C
Females (	10)																						
6	61	10	Coxarthrosis	2	1	3	3	1	1	1	1	1	1	1	3	2	2	2	2	1	1	1	1
12	45	10	Obesitas	1	1	2	2	1	2	1	2	1	1	1	2	1	1	1	1	1	1	1	2
17	49	17	Ulcus duodeni	1	2	1	3	1	1	1	2	1	1	1	3	3	2	1	1	1	1	1	1
18	60	42	Spondylosis def.	2	1	3	2	2	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1
21	49	13	Psychasthenia																				
25	60	13	Spondylosis def.	3	2	2	3	1	1	1	1	3	1	3	2	2	1	2	1	1	1	1	2
26	61	6	Arthrosis def genu amb	3	2	1	2	1	1	1	1	1	1	1	2	2	1	1	1	1	1	1	1
44	55	38	Arthralgiae	1	2	1	3	1	1	1	1	1	1	2	3	2	2	1	1	1	1	1	2
48	54	3	Spondylosis def	1	1	1	2	1	1	1	1	1	1	1	3	3		1	1	1	1	1	1
41	57	37	Spondylosis def.	2	1	2	2	1	1	1	1	1	1	1	2	2	2	1	1	1	1	1	2
Males ($n = 12$)																							
2	53	11	Spondylosis def.	1	2	3	3	2	1	1	2	1	1	3	2	3	3	1	1	1	1	1	1
3	54	2	Spondylosis def.	2	2	1	2	1	1	1	1	1	1	2	3	3	1	1	1	1	1	1	1
4	52	51	Mb Bechterew[b]	2	1	3	3	3	1	1	1	1	1	2	3	3	3		2	3	1	1	3
13	54	3	Spondylosis def	3	1	2	3	2	1	1	2	2	1	1	2	2	2	1	1	1	1	1	2
14	54	2	Spondylosis def	1	1	1	2	1	1	1	1	2	1	2	3	2	1	1	1	1	1	1	1
16	55	21	Spondylosis def.	3	2	1	3	1	1	1	1	1	1	1	1	3	1	1	1	1	1	1	1
20	57	8	Myalgiae	3	2	2	2	2	1	1	1	1	1	1	2	2	1	2	1	1	1	1	1
22	61	5	Spondylosis def																				
23	58	4	Spondylosis def	3	2	2	1	1	1	1	1	1	1	1	3	3	1	1	1	1	1	1	1
30	50	8	Spondylosis def.	1	1	3	2	2	1	1	2	1	1	1	3	2	3	1	1	2	1	1	2
49	53	12	Psychoneurosis	1	1	1	2	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	3
50	55	7	Myalgiae + Erythematos	1	2	2	2	1	1	1	1	1	1	1	2	2	1	1	1	1	1	1	1

[a] Skin biopsies lost during preparation.
[b] Excluded from the final statistical assessment.

We have considered it to be of interest to study the localization of immunoglobulins, β_{1C}-globulin, fibrinogen and albumin in skin sections in rheumatoid arthritis. Skin biopsies are easy to perform and this made it possible for us to compare the findings from a large group f rheumatoid ar thritis patients with similar findings in a control series of patients without this disease. If angiopathy in rheumatoid arthritis is disseminated in the connecti e tissue—which may be assumed from earlier studies—and the pathogenesis is similar irrespective of the vessels' locality then skin slide observations are of general interest in this disease. Comparisons can also be made with observations from other diseases with probable disseminated angiopathy such as systemic lupus erythematosus (SLE) and diabetes mellitus (12).

MATERIAL AND METHODS

The patients were hospitalized in the Rheumatological Department at the Umeå University Hospital from Oct. to Nov 1968. One group consisted of patients ith classic or definite rheumatoid arthritis according to A.R.A. diagnostic criteria (17). The other group comprised patients who were thought in all probability not to have the disease. The majority of this group ere treated for degenerative back and joint disorders (Tables I and II).

The investigation was planned from the beginning as follows. One of the team carried out the clinical assessments and took the skin biopsies. All biopsies ere numbered. The other assessed and completed the histological investigation ithout access to other data than the biopsy numbers. In this way aimed at completely "blind" investigation procedure. N attempt as made to re-examine the slides. In patients with rheumatoid arthritis we tried to assess the clinical duration of the disease. Routine blood and urine analyses (Hb g% white blood cells, differential count, SR, sugar and urine albumin, urine sediment) were made on all patients None ere known diabetics. Serum proteins ere studied ith paper electrophoresis, titre of rheumatoid factors with hemagglutination and acryl-precipitation test. The LE cell test as carried out on most patients ith rheumatoid arthritis. In none of the cases ere LE cells found.

All punch biopsies ith diameter of 3 mm were

taken from the ulnar side of the left hand abov the hypothenar musculature. The skin was anaesthetized with ethyl chloride spray. The biopsies ere fixed in 96% ethanol at +4 C for 4 hours. Details of the preparation and staining technique ha been described earlier (17). Unconjugated antisera from rabbits were obtained from Behringwerke. Antisera against the following human plasma proteins were used: albumin, β_{1c}-globulin, fibrinogen, IgA-, IgG- and IgM-globulin. Immunoelectrophoresis against human plasma gave each antiserum only one precipitation line. Only one fluorescein conjugated antiserum as used, i.e. the globulin fraction of a goat antirabbit globulin serum with predominant antibody activity against rabbit γ-globulin (Microbiol. Ass.). The slide as incubated with its respective antiserum for 30 min at room temperature (12). In order to avoid day-to-day variations in the titre strength and technique of the respective antisera, immunofluorescent staining was used so that slides from all the skin biopsies ere incubated on the same day with antiserum against albumin. Other slides were stained on the next day to demonstrate the existence of fibrinogen, etc. When studying the slides the same process as used: firstly all slides treated with anti-albumin serum were studied, and then the fibrinogen and other slides in turn. We hoped in this ay at least partly to eliminate the possibility of findings in one slide influencing our interpretation of the next slide with the same number.

Assessment of PAS staining as concentrated to the thickness of the PAS-positive essel walls in the small dermal blood essels and to the intensity of PAS staining in the border zone between dermis and epidermis. The following grading was used.

Grade 1. Normal thickness of the essel walls; dermal-epidermal junction slightly PAS-stained. Grade 2: Vessel seemed rather thicker than normal, dermal-epidermal on clearly PAS-positive seen as a continuous band. 3. A heavy thickening of the vessel all; border ne intensely red-coloured (and often appeared thicker than normal).

A similar assessment was made of the fluorescence in a fluorescent microscopical study.

Grade 1. No fluorescence or traces of eak fluorescence. Grade 2. Distinct, but not particularly bright fluorescence. Grade 3. Bright fluorescence.

The χ^2 method w used for statistical evaluation. Differences with $p>0.05$ ere assessed as not significant.

RESULTS

The results of the in estigation are shown in Tables I–III. Skin was studied from a total of 28 patients with rheumatoid arthritis (16 women, 12 men) and 21 patients with other diagnoses (10 women, 11 men). The average age for the rheumatoid arthritic patients was 52.5 years (range 26–65), the estimated mean duration was 11 years (range 1–31). The mean age of the control series was 55 years (range 45–61).

PAS staining

An assessment was made of the PAS staining in the junction between dermis and epidermis. No difference was found between the findings in the two series. A study was also made of the thickness of the PAS-positive vessel walls in the small dermal vessels (mainly capillaries and venules). Also this showed no significant difference in the results from the two series. Nor was there any positive significance regarding cell infiltration in the skin or appearance of connective tissue in the complete light microscopic study. It should be emphasized that all biopsies were taken from apparently normal skin.

Immunofluorescent staining

The general pattern of fluorescence in the skin from the different antisera against plasma proteins applied to the slides coincided in every aspect with the earlier report of Larsson (12) in an investigation on skin from the lower leg in a healthy material and patients with diabetes, rheumatic diseases and SLE. Large amounts of albumin were often seen in the extracellular connective tissue. Even in individual slides fluorescence could vary considerably within the adjacent skin regions: an increased accumulation was often seen in the sub-papillary layer and immediately below the epidermis. Occasionally there was an increased fluorescence marginally around the blood vessels, though seldom in the actual vessel walls (Tables I–III).

IgG-globulin and occasionally IgA-globulin was present in the extravascular connective tissue with approximately the same distribution as albumin. Here too fluorescence was usually most pronounced immediately below the epidermis. Often the dermal-epidermal junction was seen as a bright well-defined band. Neither was there any significant difference in the distribution of extracellular extravascular IgG-globulin between patients with and without rheumatoid arthritis. However in one patient with rheumatoid arthritis (no. 10) we did notice rows of homogeneous formations immediately below the epidermis. These formations were PAS-positive and fluoresced brightly for all investigated proteins except fibrinogen (see Discussion).

There was a significantly increased accumulation of IgG-globulin in the vessel walls in rheu-

Table III. *Result of PAS and immunofluorescence staining of skin biopsies*

N.S. = not significant ($p > 0.05$)

Histological localization of the different investigated substances	PAS and immunofluorescence staining						
	Rheumatoid (n = 28)			Non-rheumatoid (n = 19)			
	1	2	3	1	2	3	χ^2-test
Area of dermal–epidermal junction							
PAS	10	15	3	9	4	6	N.S.
Albumin	9	8	11	8	7	4	N.S.
β_{1c}-globulin	28	0	0	18	1	0	N.S.
Fibrinogen	22	3	3	16	2	1	N.S.
IgG-globulin	3	13	12	2	9	8	N.S.
IgA-globulin	16	10	2	16	3	0	N.S.
IgM-globulin	28	0	0	19	0	0	N.S.
Sub-epidermal connective tissue							
Albumin	0	16	1	1	11	7	N.S.
β_{1c}-globulin	25	3	0	19	0	0	N.S.
Fibrinogen	28	0	0	19	0	0	N.S.
IgG-globulin	0	19	9	3	10	6	N.S.
IgA-globulin	22	6	0	18	1	0	N.S.
IgM-globulin	27	1	0	19	0	0	N.S.
Vessel walls in small cutaneous blood vessels							
PAS	17	10	1	9	10	0	N.S.
Albumin	19	9	0	14	5	0	N.S.
β_{1c}-globulin	16	10	2	13	6	0	N.S.
Fibrinogen	22	4	2	14	3	2	N.S.
IgG-globulin	5	13	10	11	6	2	$0.05 > p > 0.01$
IgA-globulin	17	10	1	18	1	0	$0.01 > p > 0.001$
IgM-globulin	13	13	2	12	6	1	N.S.

Grading 1–3, see Table I.

matoid arthritis compared with the control series. Fluorescence was seen here partly between and immediately below the endothelial cells. Fluorescence was also seen in the actual cytoplasm of the cells of the vessel walls. The basement membrane of the vessels sometimes fluoresced in an even band, but more usually there was an uneven, often only partial fluorescence of the basement membrane—sometimes slightly granular—and an uneven fluorescence of the perivascular tissue. IgA-globulin was also seen in a significantly increased amount in rheumatoid arthritis. There was no significant difference in the remaining plasma proteins examined.

DISCUSSION

We have been interested in seeing whether vascular changes of diagnostic (and perhaps pathogenetic) significance could be shown in apparently normal skin from patients with rheumatoid arthritis. Earlier studies give some support to this supposition, vessel lesions have been found in muscles in this disease (18, 20) and in biopsies from rectal mucosa (19). In apparently normal skin from the lower leg Larsson (12) found in a small series a thickening of the walls in small skin vessels from patients with rheumatoid arthritis, SLE and diabetes. IgG-globulin was present in the vessel walls in all three disease groups.

In the present study no significant difference in the vessel morphology was found in PAS-stained slides. Our results coincide more or less with those of Brånemark et al. (3) electron microscopic studies of small vessels in the synovial tissue from patients with rheumatoid arthritis and from those with no inflammatory changes in

the synovialis showed no definite morphological differences. Neither could we find any connection in rheumatoid arthritis between thickening of the walls and the duration of the disease. Here we have a striking difference from the microangiopathy seen in diabetes mellitus: in this chronic disease, at least in the skin, there is a clear connection between a marked thickening of the basement membrane of the small blood vessels and the clinical duration of the disease (1?). In the immunofluorescent microscopic study a significant difference was seen between the two series. IgG-globulin and IgA-globulin were seen localized in the walls of the small vessels more often in the rheumatoid arthritis series than in the control series. Skin biopsies were taken from apparently normal skin, and as far as we know there have been no earlier reports of significant immunohistochemical differences between vessels from patients with rheumatoid arthritis and non rheumatic subjects outside the synovialis. Larsson (12) observed an increased localization of IgG-globulin to the walls of the dermal vessels in the lower leg, but there was no significant difference when compared with a series of healthy subjects. The rheumatoid group was small and heterogenous. Douglas (7) found no immunoglobulin in biopsies aken from the vessel walls of finger arterioles of umatoid arthritic patients, but he did discover lized IgG and IgM-globulin in lymph glands the same patients. Fish et al (8) and Rodman et al. (16) in a study of synovial tissue found IgG-globulin and complement in the synovial membrane in the connective tissue and in the adventitia of small vessels. IgM-globulin was observed in the cytoplasm of mononuclear cells and plasma cells. IgA-globulin was not observed. These findings from 8 patients with rheumatoid arthritis differed from those of 13 patients without this disease. Rodman et al. also found formations of extracellular localized immunoglobulin in connective tissue. These homogenous formations were similar to those observed in skin from a rheumatoid arthritis patient (no. 10) and skin from SLE patients described by Tan and Kunkel (21). Brandt et al. (?) reported the presence of IgG-globulin and β-globulin in the synovial vessel walls of 5 of 12 patients with rheumatoid arthritis, 4 of 4 with antinuclear antibodies, but negative findings in 4 patients with Mb Reiter and degenerated joint diseases. Thus in both synovial tissue and skin, IgG-globulin was found to be localized to the small vessel walls in rheumatoid arthritis. In synovialis, components of complement were shown to have the same localization and, in the skin complement was seen in the vessel walls although not in more significant quantities than in non rheumatics. We have not been able to find any connection between the presence of IgG-globulin in the vessel walls and the known duration of the disease or the activity of the disease as seen by increased SR, titre of rheumatoid factor in serum or immunoglobulin level in serum as measured with paper electrophoresis. Nor does there seem to be any such connection in the synovialis. A possible connection between the presence of immunocomplexes with IgG-globulin and complement and the development of arthritis or vasculitis is wholely speculative. However it is of interest to note that Kinsella et al. (11) found IgG-globulin and β_{1C}-globulin in the cytoplasm of most phagocytical pallisade cells in the synovial tissue. Fifteen of the 17 rheumatoid arthritis patients examined gave positive results, whilst patients with other forms of arthritis were negative. Quismorio et al. (15) reported that repeated injections of Fab-fragment from IgG-globulin in rabbit knee joints may produce chronic synovitis.

In this, as in an earlier study (12) it was seen that different plasma proteins—not only immunoglobulins but also albumin—tended to collect in the junction between the dermis and epidermis. Sometimes this region could be seen as a clear band. This has been reported earlier as a typical finding in SLE (5). None of our patients had a positive LE cell test. The reason why we found positive fluorescence in this region even in normal cases, whilst others have seen this phenomenon particularly in SLE, discoid lupus and psoriasis, is not clear. It is probably a result of differences in preparation. We used alcohol-fixed slides, most other investigators used washed quick frozen skin sections. Accumulation of IgG-globulin in the vessel walls has also been reported in SLE (17). We have not investigated this further. The often granulated or clustered distribution of fluorescence in the vessel walls in rheumatoid arthritis patients which we have observed seems to have certain similarities to the fluorescence in glomeruli reported in SLE.

It is also of interest to compare the immuno-

histological pattern of dermal vessels in rheumatoid arthritis with the corresponding skin changes in diabetes mellitus (12). In the latter disease there was a considerably more intense accumulation of fluorescence in the heavily thickened basement membrane (here too there is some granulation of the fluorescence) whilst fluorescence in rheumatoid arthritis seems to be more localized in and around the endothelial cells and even perivascularly. The similarities between the fluorescent microscopic pattern in the synovialis and dermal cells in rheumatoid arthritis may support the concept that in this disease there is a disseminated microangiopathy which partly resembles the diabetic microangiopathy but with at least certain morphological differences.

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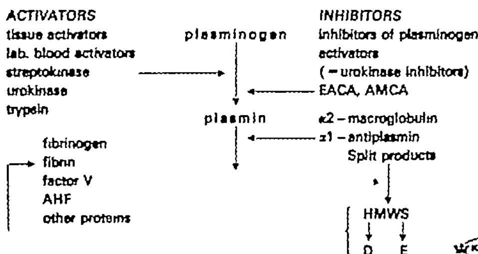
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Urinary tract haemorrhages may be caused by increased fibrinolytic activity Cyklokapron reduces or arrests fibrinolytic bleeding

In recent years fibrinolytic inhibitors have found widespread use in a number of haemorrhagic conditions, particularly in urinary tract haemorrhages and in connection with prostate surgery. Urine contains urokinase. This enzyme activates the conversion of the plasminogen present in the blood and blood clots into the proteolytic enzyme plasmin, which dissolves clots and thus sustains various types of haemorrhage in the urinary tract. Cyklokapron produces a haemostatic effect by counteracting the activity of urokinase.

The Swedish investigators, Lennart Andersson and Inga Marie Nilsson, have obtained good clinical results by administering Cyklokapron to patients suffering from haemorrhages in the upper and lower urinary tract as well as postoperative bleeding following prostate surgery. Patients suffering from haematuria as a result of general fibrinolysis were also included in the investigation. Bleeding ceased completely in all the patients in the latter group, as was the case with most of the other patients.

the fibrinolytic system



ANDERSSON L & NILSSON I M AMCA (aminomethyl cyclohexane carboxylic acid, Cyklokapron ®) potent haemostatic agent in urinary tract bleeding Scand J Urol Nephrol 3 (1969) p 183

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EFFECTS OF DDAVP A SYNTHETIC ANALOGUE OF VASOPRESSIN IN PATIENTS WITH CRANIAL DIABETES INSIPIDUS

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Abstract. DDAVP 1-deamino-8-D-arginine vasopressin, is synthetic analogue of vasopressin with higher antidiuretic potency less vasopressor activity and longer duration of action than the vasopressin preparations at present in clinical use. The effects of DDAVP have been studied in ten patients with vasopressin sensitive diabetes insipidus previously treated with 8-lysine vasopressin, chlorpropamide, chlorthalidone, or combinations of these substances. Five patients were given 1 or 2 μg DDAVP i. and the effect of these amounts lasted from 5 to more than 12 hours compared with 45–90 min for corresponding amount of 8-lysine vasopressin. In all ten patients DDAVP administered intranasally in doses f 15 μg one to three times daily normalized the urine production also in cases where previous therapy had failed. No side-effects have been observed during treatment with DDAVP for 6–12 months. It is concluded that DDAVP because of its freedom from side-effects and long duration of action, is safe and effective substance in the treatment of cranial diabetes insipidus.

Posterior pituitary extracts ha e been used in the treatment of cranial diabetes insipidus (d.i.) since 1913 (11 26) The active principle in these extracts—vasopressin—was synthesized by du Vigneaud in 1954 Because of the difficulties involved in synthesis, preparations of 8-arginine vasopressin (AVP), the hormone naturally occurring in man, have not yet been made for commercial use. How ever synthetic 8-lysine vasopressin (LVP) which is more easy to synthesize, has been commercially available for several years. In the treatment of d.i the disadvantages of these hormone preparations (side-effects and short duration of action) have led to an increasing use of other substances with antidiuretic effect. Chlorpropamide (2, 3 12, 17 18 22), thiazide preparations (8, 9), carbamazepine (5) and clofibrate (10 23) hav been used with excellent results in some cases. How ever incomplete or failing effect of these drugs occurs, and serious side-effects have been reported during treatment with chlorpropamide (20 24). Therefore there is still the need for an effec tive and safe treatment.

Synthetic analogues of vasopressin have been produced in which some of the effects of the na tural hormone are reduced and others are enhanced (4) The effects of the analogue 1-de amino-8-D-arginine vasopressin (DDAVP) make it particularly suited for the treatment of patients with vasopressin sensitive d.i. as it has a higher antidiuretic potency less vasopressor activity and a longer duration of action than AVP and LVP (25 28). The excellent results previously obtained (25) motivated further trials with DDAVP the present study reports its effects in ten patients with cranial d.i.

MATERIAL

The effects of DDAVP ere studied in ten patients, four women and six men, with cranial d.i. (Table I). The thyroid, adrenal and gonadal functions of all the patients had been investigated before the study and the diagnosis of d.i. was verified by standard clinical and laboratory methods. As Table I reveals, idiopathic d.i. was diagnosed in three cases. Seven patients had been operated on for tumours in the hypothalamic-pituitary region. Patients suf fering from panhypopituitarism were all on adequate substitution therapy All cases had earlier been treated with LVP or posterior pituitary extracts and proved to be vasopressin-sensitive.

Table I lists the previous antidiuretic therapy given to each patient. Patients 1 and 2 had normal daily amounts of urine on only LVP or posterior pituitary powder intranasally but needed 6–10 doses day This necessitated administration also during the night, and patient 2, who had suffered from his disease for more than 40 years, reported that during this time he had not slept undisturbed for single night. Six patients had only moderate effect of LVP intranasally and this preparation could not nor malize their daily urine volumes without complementary therapy One year after operation for chromophobe

Table I. *Pertinent data on the patients (the antidiuretic therapy before DDAVP given in italics)*

Pat. no	Age (y)	Sex	Diagnosis	Duration of d.i. (y)	Previous antidiuretic therapy	Additional information
1	33	♀	Idiopathic d.i.	26	Post. pituitary snuff *LVP intranasally*	Two normal pregnancies
2	57	♂	Idiopathic d	42	*Post. pituitary snuff* LVP intranasally Chlorpropamide	
3	27		Idiopathic d.i.	8	LVP intranasally *Chlorpropamide*	One normal pregnancy pregnant
4	24	♀	Panhypopituitarism D.I	7	*LVP intranasally* Chlorpropamide *Chlorthalidone* *Potassium*	Operated on at the age of 18 for craniopharyngioma
5	19	♂	Panhypopituitarism D	6	*LVP intranasally* Chlorpropamide *Chlorthalidone* *Potassium*	Operated on at the age of 13 for craniopharyngioma
6	31	♂	Panhypopituitarism D.I.	21	Post. pituitary snuff *LVP intranasally* *Chlorpropamide*	Operated on at the age of 18 for pinealoma
7	54		Panhypopituitarism D.I.	1	Pitressin tannate in oil	Operated on at the age of 53 for meningeoma
8	31	♂	Panhypopituitarism D.I.	1	Pitressin tannate in oil	Operated on at the age of 30 for chromophobe adenoma
9	40	♂	Panhypopituitarism D.i. Diabetes mellitus	7	*LVP intranasally* *Chlorpropamide*	Operated on at the age of 33 for craniopharyngioma
	46	♂	Panhypopituitarism D	4	LVP intranasally LVP subcutaneously *Chlorpropamide*	Operated on at the age of 42 for chromophobe adenoma

......m, patient 10 produced 7–10 l of urine a day. As he could not be satisfactorily controlled with intranasal LVP this preparation was given by injection. Doses which had an adequate antidiuretic effect, however, produced side-effects such as pallor, tachycardia, and desire to defecate. T patients (nos. 7 and 8) were treated with pitressin tannate oil for short period postoperatively: they responded satisfactorily.

Seven patients had been treated with chlorpropamide 250–500 mg/day. I patient this drug had no demonstrable antidiuretic effect during treatment for more than a eek. The effect was uncertain in patients 4 and 5. Patient 4 reported side-effects, particularly nausea, and did not wish to continue the treatment. Patients 6 and 9 had a moderate but not adequate effect from chlorpropamide alone, but combination of the drug with intranasal LVP reduced their daily urine volumes to about 3 l. In cases 3 and 10 chlorpropamide (250 and 375 mg day respectively) had an excellent antidiuretic effect and completely normalized the daily output of urine. Patient 3, however, had several attacks of suspected hypoglycaemia, on one occasion with convulsions at night. She also planned a pregnancy: therefore continuation of the chlorpropamide treatment was considered inadvisable. After treatment ith chlorpropamide for 18 months patient 10 developed toxicoderma; this disappeared when the treatment was discontinued.

I cases 4 and 5 the therapy consisted of combination of intranasal LVP, chlorthalidone 50 mg/day and potassium supplement. This therapy normalized the urine output in case 4, but case 5 still produced ..5–3.5 l/day.

METHODS

During the change to DDAVP treatment all patients except one (no. 10) were observed in hospital. Before the administration of DDAVP (supplied by Ferring AB, Malmö, Sweden) previous antidiuretic therapy was ithdrawn for 1 3 days in patients treated ith vasopressin only, and for 7 10 days in patients treated ith chlorpropamide or chlorthalidone. During this control period the daily water intake and rine production, as well as urine osmolality were determined.

In fi e of the patients (nos. 1 5), the effects of intravenously and intranasally administered DDAVP on diure-

Table II. *Administration of LVP and DDAVP in 5 patients during experiment days*

Pat. no.	Day of experiment	LVP intravenously (μg)	DDAVP intravenously (μg)	DDAVP intranasally (μg)
1	1	1	1	—
	2	—	—	7.5
	3	—	2	—
2	1	1	1	—
	2	—	—	15
3	1	2	2	—
	2	—	—	15
4	1	—	2	—
	2	—	—	15
5	1	—	2	—
	2	—	—	15

sis and urine osmolality were followed for 6–13 hours. Bladder catheterization was performed, and the patients were given 20 ml water/kg b.wt. during 1 hour. After that they were allowed to drink ad libitum, but to every patient an amount of water exceeding the volume of urine produced was added throughout the experiment. Urine was collected and the amounts were measured at 15-min intervals. Urine osmolality was determined in every 15-min sample by means of an osmometer (Advanced Instruments, Inc.). After three 15-min periods with constant diuresis, DDAVP in doses of 1 or 2 μg intravenously or 7.5 or 15 μg intranasally was administered. Urine was collected for further two 15-min periods and after that at intervals of 30 min. Three of the patients (nos. 1–3) were also given LVP in dose of 1 or 2 μg intravenously (0.25 and 0.5 IU, respectively) and the effects were recorded in the same way. LVP was administered before DDAVP. Only one dose of DDAVP day was tested.

During every experiment the blood pressure and the heart rate were regularly controlled, and the patients were carefully observed. Table II shows the order of these experiments.

The daily urine volumes and urine osmolalities of all patients during the control period were compared with corresponding values obtained during 3-day period when they were given 7.5 or 15 μg DDAVP intranasally one to three times daily. DDAVP was administered as solution containing 150 μg/ml by means of calibrated plastic tube ("rhinyl" Ferring AB).

All patients continued to take DDAVP intranasally after the initial treatment and have been controlled as outpatients every month or second month for 6–12 months.

RESULTS

Figs. 1–5 show the effects of intravenous DDAVP in the five patients studied. During the initial three 15-min periods the osmolalities of the urine were between 5 (case 1) and 74 (case 2) mOsm/

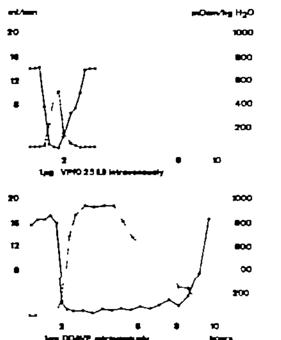


Fig. 1. Effects of 1 μg LVP and 1 μg DDAVP intravenously on diuresis and urine osmolality in patient 1. ●—● = diuresis (ml/min), ○- -○ = urine osmolality (mOsm/kg H_2O).

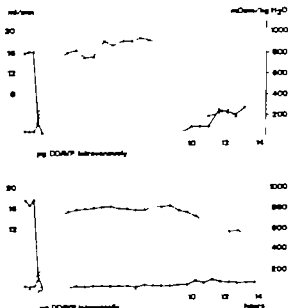


Fig. 2. Effects of 2 μg DDAVP intravenously and 7.5 μg intranasally in patient 1. Symbols as in Fig. 1.

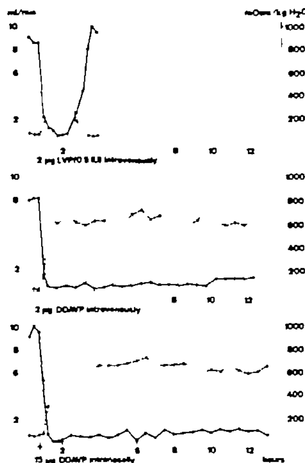


Fig. 3. Effects of g LVP and 2 μg DDAVP intravenously and 15 μg DDAVP intranasally in patient 3. ls as in Fig. 1.

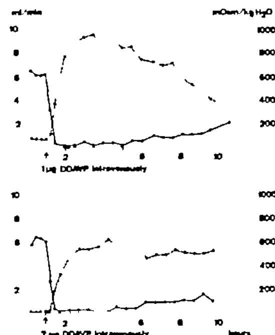


Fig. 4. Effects of 1 and 2 μg DDAVP intravenously in patients and 4, respectively. Symbols as in Fig. 1.

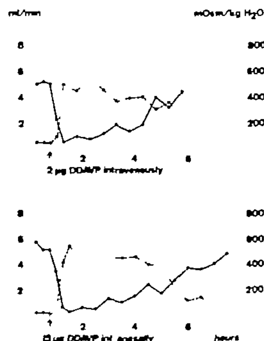


Fig. 5. Effects of 2 μg DDAVP intravenously and 15 μg intranasally in patient 5. Symbols as in Fig. 1.

kg H_2O and their urine productions varied between 5.0 (case 5) and 17.5 (case 1) ml/min. Within 15–30 min after administration of 1 or 2 μg, DDAVP suppressed the diuresis to less than 1 ml/min in all cases. During this time the osmolality of the urine began to rise; in the following periods it reached a maximum level of 550 to 955 mOsm/kg H_2O. The effects lasted from 5 to more than 12 hours. For practical reasons it was not always possible to continue the experiment to a time when the effects of DDAVP had disappeared.

In three cases in which 1 or μg of LVP were given intravenously the effects lasted only 45–90 min (Figs. 1 and 3). In patient 3, 2 μg of LVP intravenously produced pallor and mild abdominal discomfort lasting for a few minutes. No such reaction on DDAVP was observed.

Intranasally administered DDAVP at a dosage of 7.5 or 15 μg also had a prompt effect which lasted for more than 12 hours in four of the five patients studied in this way. In patient 5 DDAVP had a shorter duration of action than in the other

Table III. *Effect of DDAVP on the urine volume and osmolality in the 10 patients studied*

	Without antidiuretic therapy		DDAVP	During DDAVP therapy	
Pat. no.	Urine volume (l/day)	Urine osmolality (mOsm/kg H_2O)	dosage (µg/day)	Urine volume (l/day)	Urine osmolality (mOsm/kg H_2O)
1	17.8–19.0	40–60	7.5 2	0.9–1.7	450–780
2	12.2–14.4	75–90	15 2	1.6–1.8	510–745
3	7.0–10.0	70–135	15 2	1.1 1.3	650–685
4	5.0–6.6	50–125	15 2	1.3–1.8	340–525
5	5.3–6.3	55–165	15 3	2.0–2.5	360–410
6	6.1–7.2	125–130	15 2	0.8–1.4	500–650
7	4.2–5.3	185–225	15 2	1.0–1.2	740–850
8	4.0–6.2	25– 90	15 2	1.1 1.8	440–520
9	4.0–5.8	310–340[a]	15 2	1.5–1.8	670–680[a]
10	2.6–3.8	—	15 1	1.2 1.7	—

[a] Glucosuria.

patients, irrespective of the route of administration (Fig. 5).

By a comparison of the duration of the effects of intravenously administered amounts of DDAVP with the amounts administered intranasally the intranasal resorption in these five patients could be roughly estimated to be 10–20%.

Table III records the effect on the daily urine volume and urine osmolality in each patient. Eight of the ten patients needed 7.5 or 15 µg DDAVP twice a day to normalize the urine production. Patient 5 required 15 µg × 3 whereas in case 10 a single dose of 15 µg/day was sufficient. This patient had the lowest urine production during the control period.

In some patients a single dose of 30–45 µg/day intranasally was tried with good result, but they preferred two smaller doses a day as this dosage produced a more even diuresis.

In no case were effects on blood pressure or heart rate, pallor abdominal discomfort, or any other side-effect seen after intravenously or intranasally administered DDAVP. Nasal insufflation for more than 6–12 months has shown a fully satisfactory effect without reactions from the nasal mucosa. In no patient has a decrease of the effect of DDAVP so far been observed.

DISCUSSION

Injection therapy with vasopressin preparations often produces side-effects such as pallor nausea, increased tonus of the intestines and bladder with abdominal pains, a desire to defecate and sometimes diarrhoea. Cardiac complications in patients with coronary heart disease have also been reported (21). Intranasal administration of posterior pituitary snuff often causes rhinitis, but this complication can be avoided by using synthetic AVP and LVP preparations, which to an increasing extent have replaced posterior pituitary extracts in the treatment of d.i. A disadvantage of these preparations is their short duration of action (2–4 hours) which necessitates frequent administration (also at night). Long-acting preparations, such as pitressin tannate in oil, have an effect for 1–2 days in favourable cases, but the injections which should be given intragluteally may be painful and the effect is difficult to control.

Since the first report of its effect in cranial d.i. (3) chlorpropamide has attained an important therapeutic role (6). In some cases of vasopressin sensitive d.i., however chlorpropamide has no, or only a moderate effect (13 16) and several side effects, particularly hypoglycaemia, have been reported (19 27). Discussions about possible harmful effects of chlorpropamide on the foetus (15) contraindicate its use during pregnancy. The disadvantages mentioned were encountered also in the patients presented in this study. Out of seven patients treated, moderate or doubtful effects were seen in four and in one patient the drug was ineffective. Treatment had to be discontinued because of attacks of hypoglycaemia or toxicodermia in the two cases in which it had an excellent antidiuretic action.

Thiazides are effective in both renal and cranial d.i. (8 9). As these preparations often have only a moderate effect in cases of cranial d.i., and

hypopotassaemia is a common side-effect, they are mainly used as a complement to other kinds of treatment.

The substances recently introduced as antidiuretics carbamazepine (Tegretol®) and clofibrate (Atromidin®) are reported to have a good effect in some cases of vasopressin-sensitive d.i. and very few side-effects (5, 10). Experiences of these drugs as antidiuretics are limited, but failures in treatment of vasopressin-sensitive d.i. have been reported (20). It has been suggested that their mode of action is similar to that of chlorpropamide (24).

In the treatment of d.i. caused by a lack of vasopressin it is most satisfactory to replace the failing hormone. By modifications of the vasopressin molecule it is possible to enhance some of the effects of vasopressin and at the same time to reduce or eliminate others. Deamination in position 1 (cys[1]) often increases the ratio antidiuretic/vasopressor effect by enhancement of the antidiuretic activity (14). It has also been shown that replacement of L-forms of amino acids in the natural hormones, with D-forms in position 8 is usually associated with a reduction of the pressor effects of vasopressin (28, 29). Because of deamination in position 1 and replacement of L- inine in the natural hormone by D-arginine, AVP has two clinically useful effects which both reflected in the present results: an in- eased antidiuretic/vasopressor ratio and a prolongation of action.

The antidiuretic/vasopressor ratio for AVP is 1 and that for LVP is very close to 1 (4). The corresponding ratio for DDAVP is 79 at very low dosage, but at higher dosages the ratio is increased to 2 500–4 500 (25). Even at a high dosage of DDAVP the risk of pressor reactions in d.i. patients, therefore, seems negligible. Such interpretation is suggested by results in healthy volunteers who were given up to 16 μg of DDAVP intravenously as a single injection without any side effects (1). DDAVP compared with LVP has a very prolonged antidiuretic effect, which has also clearly been shown in the present study. This may be due to several factors. It is probable that the increased antidiuretic potency is of importance but it is also possible that the presence of a D-isomer in position 8 delays the normal enzymatic degradation of DDAVP in the tissues, as suggested by Vávra et al. (25).

All patients in the present study responded satisfactorily to intranasally administered DDAVP. The degree of resorption after intranasal application is difficult to estimate but probably in the range of 10–20%. This means that the effective maintenance dosage for most patients was 3–6 μg/day.

Compared to chlorpropamide, DDAVP has the advantage of having no side-effects. During a follow-up of 6–12 months DDAVP in intranasal administration proved to be adequately effective in all cases of vasopressin-sensitive d.i. so far investigated, and there has been no need for complementary therapy.

Patients with cranial d.i. and insufficiency of the anterior pituitary gland have been reported to be especially sensitive to the hypoglycaemic action of chlorpropamide (16, 27). In these cases DDAVP offers a safe alternative treatment. Pregnancy does not contraindicate the use of this preparation (7).

In our patients treatment with DDAVP proved to be more effective than the therapy previously offered. DDAVP is easy to administer and, because of its freedom from side-effects, we have also found it to be a safe preparation.

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HYPER-α-LIPOPROTEINEMIA IN MEN EXPOSED TO CHLORINATED HYDROCARBON PESTICIDES

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Abstract. Twenty-two male subjects exposed in their occupation to a mixture of chlorinated pesticides, mainly lindane and DDT and 19 healthy males have been studied with regard to their fasting serum lipid levels and to the amounts of lipids in the three ultracentrifugally separated lipoprotein families, very low density (VLDL or pre-β), low density (LDL or β) and high density (HDL or α) lipoproteins. Six exposed subjects had hypertriglyceridemia (type IV pattern) and one hypercholesterolemia (type II A). Forty per cent of the subjects exposed to chlorinated pesticides and none of the controls had hyper HDL(α)-lipoproteinemia. In two of these subjects the level of HDL(α)-lipoprotein was about two to three times above average normal values. The possible cause of the frequent occurrence of this rare type of hyperlipoproteinemia after exposure to pesticides is discussed.

A common concept is that chlorinated hydrocarbon pesticides are transported in blood dissolved in the plasma triglycerides (8). Also a binding of these pesticides to β-lipoprotein has been *suggested by Lockens (14). Their lipid affinity is* further suggested by the fact that the chlorinated pesticides accumulate in adipose tissue (8). Against this background we decided to investigate various aspects of plasma lipoprotein metabolism in subjects exposed to chlorinated pesticides (12). In this study we report the finding of a high incidence of hyper-α-lipoproteinemia in such persons.

MATERIAL

The study includes 22 men from Stockholm, exposed occupationally daily or at least weekly to a mixture of pesticides, mainly lindane and DDT. They worked with the pesticides in spray form, so that both skin exposure and inhalation were possible. All were healthy clinically. Two groups of subjects were included as non-exposed healthy controls for the lipoprotein concentration. One group has been described previously and consisted of healthy males in the age range 25–70 years (3). The amount of α-lipoprotein in plasma of these men was determined after separation of the lipoproteins by chromatography on glass beads (4). The other group consisted of 19 healthy males from Stockholm, aged 39–65 years. From the latter blood was obtained in the fasting state and the serum lipoproteins were separated and analysed by the same methods as for the exposed men.

METHODS

Blood was withdrawn by venipuncture in the morning after fasting overnight and allowed to clot at room temperature. Serum was recovered by centrifugation within 1–3 hours after withdrawal of blood. To each ml of serum 0.01 ml of 5% Na-EDTA was added to give a final EDTA concentration of 0.05%. The serum was then stored at +4°C. Paper-electrophoresis according to the method of Lees and Hatch (13) was started on the day of blood drawing. Separation of the serum lipoprotein into *very low density* (VLDL, $d < 1.006$), *low density* (LDL, $d = 1.006 - 1.063$) and *high density* (HDL, $d > 1.063$) lipoprotein families was done not later than 14 days after sampling and performed, as described previously in a Spinco preparative ultracentrifuge (5). Analysis of the cholesterol and triglyceride content of the isolated lipoprotein families and of whole serum was done by a semi-automatic technique in a Technicon Autoanalyzer (2, 10). The phospholipid concentration was determined in some of the isolated lipoprotein families as described previously (3).

RESULTS

The results of the study are given in Table I for each subject. Five subjects had hypertriglyceridemia with serum levels above 2 mmol/l at the first analysis. These subjects all had increased levels of the VLDL (pre-β) lipoprotein family and their lipoprotein pattern corresponded to type IV

Table I. Age, serum total triglycerides (TG) and cholesterol (Chol) triglyceride and cholesterol content of the three ultracentrifugal lipoprotein families and phospholipid (Phosph) content of the HDL-lipoproteins and lipoprotein types according to Fredricksson et al. as modified (1) in the 22 exposed subjects

		Serum lipoprotein families										
		Serum		VLDL		LDL		HDL				
Subject no.	Age (y.)	TG (mmol/l)	Chol (mg/100 ml)	TG (mmol/l)	Chol (mg/100 ml)	TG (mmol/l)	Chol (mg/100 ml)	TG (mmol/l)	Chol (mg/100 ml)	Phosph (mg/100 ml)	Type[a]
1	21	0.85	148	0.39	9	0.30	78	0.31	51	—	N
	28	0.79	167	0.32	8	0.31	95	0.20	60	125	N
3	31	0.79	215	0.46	11	0.28	153	0.14	61	120	N
4	33	1.37	301	0.77	22	0.48	236	0.25	74	140	II A, hyper-α
5	33	1.70	222	1.04	30	0.38	120	0.27	72	173	N hyper-α
6	35	1.90	251	1.18	27	0.48	166	0.23	58	86	N
7	40	3.76	290	[illegible].34	57	0.68	145	0.31	38	83	IV
8	40	1.37	242	0.77	17	0.41	167	0.14	70	165	N hyper
9	44	2.12	251	1.41	25	0.49	187	0.27	67	147	IV hyper-α
10	49	0.89	280	0.34	5	0.30	101	0.11	148	295	N, hyper-α
11	51	1.85	236	1.17	24	0.47	168	0.23	52	136	N
12	52	1.17	258	0.57	17	0.36	173	0.17	86	167	N, hyper
13	52	2.74	243	1.79	33	0.59	162	0.35	48	118	IV
14	52	1.01	244	0.42	14	0.41	142	0.15	71	—	N
15	54	[illegible].88	210	1.94	63	0.40	74	0.27	47	1[illegible]4	IV
16	56	0.98	237	0.45	12	0.43	1[illegible]4	0.11	86	—	N hyper-α
17	57	1.45	252	0.72	19	0.52	161	0.29	73	144	N
18	62	1.61	240	0.98	23	0.42	163	0.23	44	81	N
19	64	1.76	260	1.01	14	0.42	120	0.23	103	257	IV hyper-α
20	65	1.43	245	0.76	16	0.47	173	0.23	71	175	N hyper-α
21	66	1.20	227	0.73	10	0.30	169	0.14	58	126	N
22	70	[illegible].53	262	1.36	31	0.24	160	0.22	46	118	IV
Repeated analyses											
	33	1.58	298	0.98	21	0.39	223	0.22	64	—	II A
	33	1.77	213	1.16	31	0.35	103	0.34	86	—	IV hyper
	49	[illegible].04	208	1.59	21	0.28	91	0.25	112	—	IV hyper
	52	0.87	237	0.39	9	0.34	170	0.13	77	—	N hyper
	56	0.72	237	0.23	8	0.40	162	0.11	88	—	N hyper
7	57	1.47	219	1.01	14	0.33	142	0.19	66	—	N

N = normal.

pattern according to Fredrickson et al. (7). Abnormal lipoprotein pattern can be typed as I, II A, II B, III, IV and V (1). One of the men had slight hypercholesterolemia with raised amounts of LDL (β) of the type II A pattern.

The α-lipoprotein band was quite pronounced on the lipoprotein paper-electrophoresis in several of the exposed subjects, and the amount of cholesterol and phospholipids recovered in the HDL (α) family was often higher than is normally seen. In order to establish how many subjects had abnormally high levels of HDL (α) previously established normal limits were used (3). As at that time these normal limits were determined by another method than ultracentrifugation, i.e. by chromatography on glass beads (4) we also include ultracentrifugal HDL (α) values from male control subjects who were apparently healthy and had not been exposed to chlorinated pesticides (Figs. 1 and 2). As both the cholesterol and the phospholipid concentration had been seen to increase linearly with age (3) the HDL (α) values are plotted against age. Fig. 1 reveals that 6 of the exposed subjects had HDL (α) cholesterol above normal range, but none of the control subjects. For HDL (α) phospholipid 8 exposed subjects were above the normal range: once again controls were all below the previously established upper normal range (Fig. 2). Altogether 9 of the 22 exposed subjects had an elevation of HDL ( ) cholesterol and/or phospholipid. Five subjects had elevation of both cholesterol and phospholipids,

one of cholesterol only and three of the phospholipid content. Of the subjects with elevated HDL ( ) levels two had values about two to three times the normal amount of both cholesterol and phospholipids.

Three of the 9 subjects who had hyper-α-lipoproteinemia had in addition other lipoprotein abnormalities (*II A and IV*) (Table I).

We had the opportunity to repeat the analysis in 6 subjects (Table I). Four of these were originally classified as ha ing hyper-α-lipoproteinemia. This was also the case in 3 of them at the second study. Two of the 6 were considered normal on the first occasion and, on the second, one was classified as ha ing hyper-α-lipoproteinemia.

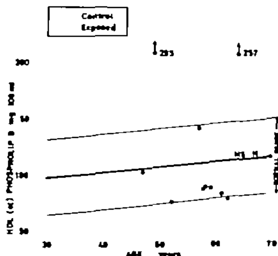


Fig. 2. HDL (α) phospholipid and age for exposed and control subjects. The normal mean and range according to Carlson (4).

DISCUSSION

Hyper HDL(α)-lipoproteinemia is a rare lipoprotein abnormality. Primary (genetic) forms of hyper HDL( )-lipoproteinemia are not known (6). Secondary hyper-α-lipoproteinemia has been described. Considerably increased amounts of HDL occur in biliary obstruction, notably biliary cirrhosis (6). These lipoproteins, although they behave as HDL in the preparative ultracentrifuge, differ in several other respects from the HDL family. They have a higher cholesterol/phospholipid ratio approaching 1 (6), while the normal is around 0.5 (3). Furthermore they do not migrate as -lipoproteins on paper-electrophoresis. The subjects exposed to pesticides with increased HDL had a fairly normal cholesterol/phospholipid ratio and their lipoprotein migrated as -lipoprotein on paper-electrophoresis. It thus seems unlikely that the raised HDL ( ) was due to biliary obstruction. High levels of α-lipoproteins ha e been seen in severe alcoholics (9). In such subjects it seems as if the lipoprotein abnormality bears the closest relationship, if any, to malnutrition (9). We had no evidence of severe alcoholism in our subjects. Kneidel (11) has reported liver damage with increased serum GOT and GPT in men xposed to benzene hexachloride. Radomski et al. (15) reported higher levels of DDT and its analogues in *autopsy material with the pathological diagnosis* of portal cirrhosis and hypertonia. However, the persons examined in their study had normal serum GOT and GPT.

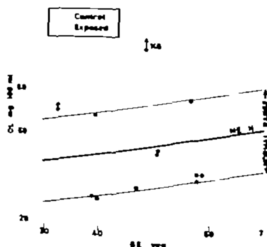


Fig. 1. HDL (α) cholesterol and age for exposed and control subjects. The normal mean and range according to Carlson (4).

Kolmodin et al. (12) reported a shortened plasma antipyrine half-life in the workers examined in their study, suggesting induction of the liver microsomal enzymes. The possible relationship of this finding to hyper-α-lipoproteinemia is unknown.

The changes reported in the present investigation may reflect a transport mechanism for pesticides or may suggest a subclinical liver affection. Pesticide analyses in the various lipoprotein families are in progress (Kolmodin and Carlson).

ACKNOWLEDGEMENTS

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DISPLACEMENT AND FRACTURE OF PACEMAKER ELECTRODE DURING PHYSICAL EXERTION

Report on Three Cases

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Abstract. Three patients with electrode failure arising during permanent pacemaker treatment for AV block are discussed. The physical capacity of all three was improved after pacemaker implantation. In patients 1 and 3 electrode displacement took place during apple picking and lobster fishing. In patient 2 pacemaker implantation was carried out in connection with the insertion of an aortic ball valve prosthesis. He has later been in functional class I. Electrode fracture took place while he was carrying a 50-kg flour sack, the central fragment of the electrode being displaced to the right ventricle, where it has remained coiled up for 2 years, the battery and peripheral fragment of the electrode having been removed. Patients with good cardiac function after pacemaker implantation should therefore be warned against vigorous physical exertion which may directly affect the pacemaker system.

The most frequent complications in permanent pacemaker treatment are due to electrode or pacemaker failure. Increased experience is making purely surgical complications rarer (7).

Less frequent complications are adhesive endocarditis, myocardial perforation and dissection and the formation of thrombi in the right atrium and ventricle with pulmonary embolism (2, 3, 4, 5, 6, 9, 10). Electrode fracture, with displacement of the catheter to the right atrium and ventricle is rare (1, 8).

An account will be given of three patients in whom electrode failure occurred during exertion.

CASE REPORTS

Case 1

Farmer's wife, born in 1899. Several Adams-Stokes attacks, starting in 1960. An AV block occurred and on June 24, 1966, a pacemaker (Elema 139) was implanted in the left pectoral region, connected to an endocardial unipolar electrode and an indifferent subcutaneous electrode.

An X-ray taken on July 8, 1966, showed that the intracardial electrode had become displaced in a somewhat medial direction in the right ventricle. The pacemaker function was satisfactory. On Sept. 25, 1966, while the patient was picking apples, she almost fell out of the tree, which she clutched with her left arm. This gave her a strong jerk in the arm, and she later noted a tendency to fainting spells. When hospitalized on Sept. 28, 1966, her heart rate was 40/min. ECG revealed partial failure of the pacemaker function, and fluoroscopy that the tip of the electrode was displaced to the tricuspidal orifice. On Oct. 1, 1966, a new endocardial electrode was implanted and connected to the same pulse generator. The pacemaker then functioned satisfactorily until July 1967, when the pulse generator had to be changed due to fracture of the battery. The pulse generator has since been changed twice, the last time in Aug. 1970. Her cardiac status has in the last 5 years been good (N.Y. H. A. class II).

Case 2

Farmer, born in 1916, admitted to the hospital in 1965 suffering from syncopal episodes and dizziness on physical effort. BP 110–170/70–90 mmHg. Heart pulse rate 60, regular. The heart was enlarged. A systolic ejection sound, and a weak diastolic murmur along the left edge of the sternum could be heard on auscultation. ECG showed 1st degree AV block. Two unsuccessful attempts at heart catheterization were made: the first resulted in ventricular fibrillation, which was electro-converted, and the second in perforation of the pericardium.

In Sept. 1967 ECG showed alternating 1st and 2nd degree AV block with Wenckebach's periodicity. Heart size had increased from 500 to 990 ml/m²

On Oct. 4, 1967, the aortic valve was resected and a Starr-Edwards valve no. 11 implanted. An AV block occurred during surgery and a temporary pacemaker electrode was inserted. As the block persisted, an endocardial fixed rate pacemaker system (Ventricor) was im-



Fig 1 Case 2. March 1969 X-ray of thorax in posterior-anterior position showing pacemaker and the two fragments of the electrode, the central fragment in the right ventricle. The electrode is marked in ink. The aortic ball valve prosthesis is not visible.

lanted one week later The heart rhythm subsequently ... ged to 1st degree AV block and interference occurred. ... Oct. 31 1967 an endocardial demand pacemaker ... em (Ectocor) was implanted, with pulse generator in ... right pectoral region. His recovery was complicated by febrile reaction of long duration, considered to be a postperfusion syndrome. There were slight signs of hemolysis, caused by the aortic ball valve prosthesis.

A check-up in March 1968 showed the clinical state to be satisfactory and pacemaker function normal. Heart volume had regressed from 950 to 675 ml/m² The patient subsequently worked full-time on his farm until the end of Feb. 1969 when he carried 50-kg flour sack on his right shoulder A spasmodic stimulation of the pectoral muscles occurred in connection with this, but troubled the patient so little that he waited 3 weeks before consulting doctor

On examination on March 11 1969 regular muscular spasms were occurring in the right pper extremity at a rate of 66/min. Heart rate 54. ECG showed a sinus rhythm and failing pacemaker function. X-rays showed electrode fracture, part of the electrode being coiled up in the right entricle (Fig. 1) The pacemaker and the peripheral fragment of the lectrode were removed, but it was not considered that removal of the intracardial part of the electrode was indicated. The patient has subsequently been working full-time (functional class I). Since the aortic valve surgery the patient has been treated with anticoagulants. Check-up on June 4, 1971, showed normal function of the aortic ball valve prosthesis. No sign of cardiac failure. ECG showed sinus rhythm. X-rays showed electrode in same position (Fig. 2).

Hemolysis examination showed urobilinuria + 1/50. Hb 12.7 g/100 ml, erythrocytes 4.0 mill., reticulocytes 3 ‰. bilirubin 0.97 mg/100 ml. HBDH 543 U/l. LDH 300 U l. Haptoglobin 0. Serum iron 184 µg/100 ml. TIBC 360 µg/100 ml. Thrombotest 7%.

Case 3

Fisherman, born in 1898. Due to total AV block an endocardial pacemaker system (Ventricor) was implanted on July 22, 1969 with pulse generator in right pectoral region. On Oct. 18, 1969 after having pulled lobster pots p from a depth of 10–20 m, he became dizzy and his pulse dropped from 72 to 42. On hospitalization on the same day varying heart/pulse rate of 43–68 as found. ECG showed partial failure of pacemaker function, every 3rd or 4th potential not leading to ventricle depolarization X-rays of thorax on tw planes did not confirm displacement of electrode. For 6 days the pulse rate varied f om 36 to 72, normal pacemaker rhythm being then established at 72 min. The pacemaker later functioned satisfactorily but on May 30, 1970, new pacemaker system (Ventricor) had to be inserted due to wound infection The pulse generator was implanted in the left pectoral region. The old electrode as allowed to remain in the right ventricle. The pacemaker has since functioned normally



Fig. 2. Case 2. June 1971. X-ray of thorax in left anterior-oblique position showing the electrode in the same position in the right ventricle.

DISCUSSION

After implantation of a pacemaker the cardiac function of all three patients improved so much that they engaged in heavy physical work, which was in all three cases the direct cause of pacemaker failure.

Case 2, who has an electrode catheter in the right ventricle, has been followed up for more than 2 years. H has had no symptoms attribut e to this and is working full-time. There is no clinical sign that the catheter has influenced his dynamic status. Prophylactic anticoagulant tment is important, as there is a considerable nger of thrombus formation in this patient, who two foreign bodies in his heart.

The electrode represents a danger of infection perforation. On the fluoroscopy screen the en end of the catheter could be seen striking ventricle wall during the systole.

The patient has moderate anemia, caused by intravasal hemolysis. Hemolytic activity has not increased since the electrode fracture and may probably be ascribed mainly to the aortic ball valve prosthesis.

We have considered removing the catheter but feel that open heart surgery is too risky in this patient, as earlier major heart surgery was followed by serious complications. Falcbuk et al. (1) used a transvenous technique to remove an electrode from the right atrium and ventricle by means of a Lehman catheter. Pappas et al. (8) advise against this procedure, as one cannot be sure that the catheter is endothelialized, and as there is a risk of dislodging thrombi.

These case records show that patients with pacemakers should be advised against too igorous *movements of the upper extremities or a direct* load on the shoulder region where the electrode catheter runs, due to the danger of displacement or fracture. Patients with physically exacting work and good cardiac function are particularly ex posed to such complications.

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VIRUS ANTIBODY LEVELS IN RHEUMATOID ARTHRITIS AND SYSTEMIC LUPUS ERYTHEMATOSUS

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Abstract. Sera from 30 patients with rheumatoid arthritis (RA), 10 with systemic lupus erythematosus (SLE) and 99 healthy control subjects have been studied for several viral antibodies by haemagglutination inhibition (HI) and complement fixation (CF). Measles HI antibodies were significantly increased in both RA and SLE. The geometric mean of the measles antibody titres, expressed as $\log_2$ of the reciprocal mean antibody titre, wher 1.00 equals titre of 1:10, was 1.17 in the controls, 2.45 in patients with RA ($p < 0.01$), and even higher, and 3.80 ($p < 0.01$), in patients with SLE. The measles CF antibody titres of SLE patients, but not of RA patients, also differed significantly from those of controls. In addition, the mean rubella HI titre was slightly increased in SLE ($p < 0.02$). The significantly elevated measles antibody levels in RA and SLE may be due to altered immunological reactivity in these diseases, but viral aetiology cannot be excluded.

The possible viral aetiology of connective tissue diseases has been the subject of considerable interest during the last few years. Electron micrographs show virus-like structures in the cytoplasm of endothelial cells in renal biopsy specimens from patients with systemic lupus erythematosus (SLE). The structure of these inclusions resembles the inner helix of a paramyxovirus (3, 5, 8, 9, 11). There is also suggestive evidence of a possible role of virus infection in rheumatoid arthritis (RA). Persistent differences between normal and rheumatoid synovial membrane cells, such as differences in morphology, life span in culture, certain biochemical and immunological properties, may well be the result of a slow virus infection (12). Warren et al. (15) reported the induction of transmissible acute and chronic polyarthritis in mice and their litters by injections of synovial tissue obtained from RA patients. This suggests the presence of an active infectious agent or factor in these tissues. More recently Grayzel (4) has reported that rubella virus induces a cytopathic effect and death in normal cells in cell culture within 10–14 days, while rheumatoid synovial membrane cells are not visibly affected.

The findings above are of interest in view of the study of Phillips and Christian (10) who found increased measles and parainfluenza type I haemagglutination inhibition (HI) titres in patients with SLE and Reiter's syndrome. This finding has been confirmed by others and, in addition, elevated antibody titres to other viruses have been observed in SLE (1, 6, 7, 13).

In the present study patients with definite RA, with SLE, and healthy control subjects have been tested for antibodies to several viral antigens by HI and complement fixation (CF) tests.

MATERIAL AND METHODS

Clinical material

The series consisted of 30 patients with RA and 10 patients with SLE treated in the Departments of Medicine, Helsinki University Central Hospital, or in the Department for Rheumatic Diseases at Kivelä Hospital, Helsinki. All the patients fulfilled the criteria for definite classical RA and for definite SLE, respectively. Patients with RA had effusion at least in one joint and symptoms in several joints. Tests for rheumatoid factor (Waaler-Rose and latex) were positive. Patients with SLE had the characteristic symptom complex of SLE of multiple system involvement. All the patients had positive tests for LE phenomenon and antinuclear antibodies. Patients with RA and SLE were admitted to the hospitals because of acute symptoms, and therefore only cases with acute disease are included in the series. Sera from 99 control subjects were obtained from the Finnish Red Cross Blood Transfusion Centre. The control group was chosen to match the RA and SLE patients for age and sex.

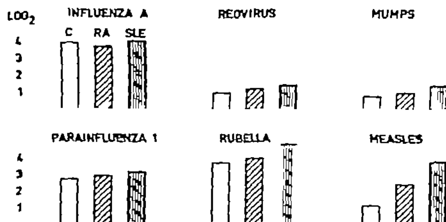


Fig. 1. Mean HI antibody titres to some viral antigens in healthy control subjects (C), RA patients and SLE patients. Vertical lines represent $\log_2$ of the reciprocal antibody titre, where 1.00 equals a titre of 1 : 10.

Haemagglutination antigens

In the HI titrations the following viral antigens were used. Measles: tween 80- and ether-treated supernatant fluid of measles-infected U cell (continuous human amnion cell line) culture. Rubella: tween 80- and ether-treated supernatant fluid of rubella-infected BHK-21 cell suspension culture. Influenza A 2: haemagglutinin purified from virus-containing allantoic fluid by gradient centrifugation. Mumps: virus-containing allantoic fluid. Reovirus: virus-containing U cell homogenate.

CF antigens

Herpes simplex: virus-infected BSC-1 cell homogenate. Cytomegalo: homogenate of virus-infected primary human embryonic fibroblasts. Rubella: virus-infected BHK 21 cell homogenate centrifuged at 30 000 rpm for 90 min in a Spinco SW 39 rotor to remove the virus particles. Measles: homogenate of virus-infected VERO cells treated with tween 80 and ether. Influenza A 2 S and mumps S: homogenate of chorioallantoic membranes from virus-infected cells. Control antigens: homogenate of uninfected BHK-21 and VERO cells, normal allantoic fluid; homogenate of chorioallantoic membranes from chick embryos.

Pretreatment of sera and titrations

HI titrations: kaolin-treated and RBC absorbed. Chicken RBC used in rubella, mumps and para 1, human O-cells in influenza A 2 and reovirus, and rhesus monkey RBC for measles. CF titrations: heat inactivation of sera for 30 min at 56°C. Micromethod was used in all HI and CF titrations.

Statistical analysis

Statistical analysis was carried out by dividing the antibody titres into high and low titres at the median point of the normal controls and also at points above and below it. The high and low titres were compared using the χ^2 test.

RESULTS

The geometric means of the HI antibody titres to 6 different viral antigens are shown in Fig. 1. The patients with RA and SLE did not differ significantly from controls in their HI titres against influenza A 2, reovirus, mumps or parainfluenza 1 antigens. HI titres against rubella were slightly elevated in SLE ($p < 0.02$), whereas the difference in the geometric mean titres between RA and control subjects was not significant. In contrast, the measles antibody titres of patients with RA and SLE were significantly increased. The geometric mean of the measles HI titre, expressed as $\log_2$ of the reciprocal antibody titre, where 1.00 equals a titre of 1 : 10, was 1.17 in control subjects, 2.45 in RA ($p < 0.01$) and 3.80 in SLE ($p < 0.01$). The number of positive specimens and the distribution of positive measles antibody titres were very different in the three groups (Fig. 2). Only one patient with SLE showed a titre of less than 1 : 10 and all the patients with RA reacted at a dilution of 1 : 10 or greater. Of the controls, 26 subjects were nonreactive even at this lowest dilution. Eight of the 10 SLE sera reacted at a dilution of 1 : 80 or greater. The corresponding figure for RA was 15 out of 30, while only 19 of 98 control subjects reacted at 1 : 80 or higher dilutions.

The range and distribution of CF antibodies against influenza A 2, mumps, rubella, reovirus, polio 1 and respiratory syncytial virus antigens were essentially similar in RA, SLE and control groups. With the exception of occasional high values in SLE this was also true of CF antibodies against cytomegalo and herpes simplex virus antigens. The mean geometric titre of measles CF antibodies was not elevated in RA, but some highly elevated titres were detected (Fig. 3). Again the titres in SLE patients were higher than in the controls ($p < 0.02$). Three very high titres of more than 1 : 256 (1 : 10 240, 1 : 20 480, and 1 : 20 480) were detected, while the 98 control

subjects had measles CF titres of 1 128 (one case) or less.

Several factors were tested to find out if they affected the observed differences in measles antibodies between controls and patients with SLE and RA. Age, sex, duration of the disease, intensity of the symptoms or therapy had no consistent correlation with antibody titres. Two patients out of three with very high measles CF antibodies also showed reactivity against control antigen, but the titres were low compared to the viral antibody titres. With the exception of these two cases and one RA patient, no patients or control subjects showed reactivity against control antigen preparation The total IgG concentrations of the sera tested with radial immunodiffusion plates (Orion, Helsinki, Finland) revealed no significant differences between the three groups. Neither was any correlation observed between the elevated antibody titres to measles virus and antinuclear antibody titres (determined by the indirect immunofluorescence technique) The sera of the SLE patients were fractionated on a sucrose gradient to separate 19S and 7S antibodies (14) and the fractions were tested for measles, rubella, parainfluenza and mumps HI antibodies. In all cases the viral HI antibodies were in the 7S fractions, and no evidence of the presence of IgM antibodies was found.

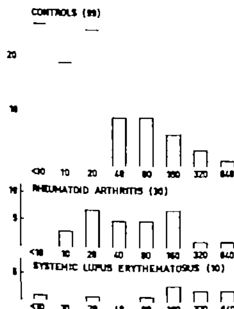


Fig. 2. Distribution of measles HI antibody titres in healthy control subjects, RA patients and SLE patients. Vertical lines represent the number of subjects and horizontal lines the measles antibody titres.

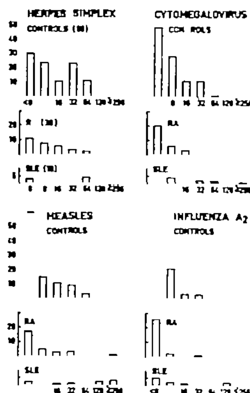


Fig. 3. Distribution of CF antibody titres to different viral antigens in healthy control subjects, RA patients and SLE patients. Vertical lines represent the number of subjects and horizontal lines the viral antibody titres.

DISCUSSION

In agreement with the studies by Phillips and Christian (10) and Hollinger et al. (6) the results of this study showed elevated measles antibody titres in SLE in both HI and CF tests. In addition, the measles HI antibodies were significantly elevated in the sera of patients with definite RA, even if the mean geometric titre for measles antibodies in RA was lower than in SLE. It has recently been reported that SLE patients have increased virus antibody levels not only against measles but also against rubella (1 6, 7 13) parainfluenza type 1 (1 6, 7 10) parainfluenza type 2 and 3 (6) mumps (6), reovirus 2 (6) respiratory syncytial (7) infectious bronchitis OC43 (7), herpes (7) and Epstein-Barr viruses (1) In previous studies (6, 10) it has also been shown that patients with some other connective tissue disorders (in-

cluding RA, polymyositis, polyarteritis, and scleroderma) tend to have increased antibody titres against several viral antigens.

For several reasons the conclusions on the pathogenetic role of the virus in connective tissue diseases must be viewed with caution. The increased antibodies were not specific for any one virus, nor was an increase in virus antibody specific for any one disease. Until now attempts to isolate viruses from biopsy material obtained from tissues of patients with RA and SLE have been unsuccessful (2, 12). Viral antibody titres, on the other hand, may be elevated in a non-specific way. The disease may alter the humoral antibody mechanism so that certain virus infections produce stronger antibody responses. The diseases may also make a patient more susceptible to some viral infections. High antibody titres to both RNA and DNA viruses have been found. This also suggests that some unspecific mechanism may be involved.

Nevertheless, for several reasons a virus infection as an aetiological factor in connective tissue diseases remains a possibility. Some indirect evidence of persistent viral infection has already been presented in the introduction. A virus infection could also explain the widespread inflammation, cell destruction and antibody formation to ious cell components, which are characteristic collagen diseases. Even if there seem to be nificant elevations of various virus antibodies in RA and SLE, there are great differences between the intensity of the antibody response to different viral antigens. This might be because only one of these viruses has a pathogenetic effect and the elevated antibody responses to other viral antigens merely reflect cross-reactions. As no known virus is clearly a better candidate than the others, there is also the possibility that an as yet unidentified agent, which shares antigenic properties with the tested viruses, lies behind the condition. Furthermore, with the possible exception of reovirus, the increased antibody titres have all been against enveloped viruses which mature at the host cell membranes. The envelopes of these viruses are partly formed from the cellular membrane structures and a virus infection may modify the membrane material. This could lead to an immune response against the antigenically modified cell membranes. Consequently even if the present knowledge of the significance of a possible virus infection in RA and SLE is scanty there may be reasons for continuing to try to determine the role of virus infection in connective tissue diseases.

ACKNOWLEDGEMENT

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IDIOPATHIC SCOLIOSIS IN OLD AGE

II. *Cardiovascular Function*

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Abstract. Cardiovascular function has been studied in female patients, 56 to 63 years of age, who were 'lagnosed as idiopathic scoliotics about 50 years ago. ght of the patients had severe skeletal deformity with spinal angle of ≥60° (SD group); the remaining seven tients were less deformed (LD group). The examinations ided ECGs at rest and during exercise, determination f work capacity total amount of hemoglobin, blood ol- and heart volume. Through heart catheterization 13 subjects intracardiac and intravascular pressures cardiac output were measured at rest and during Pulmonary hypertension with restricted orking capacity was found in the most severely deformed patient; all others had ordinary pressures and cardiac output both at rest and during work when compared with healthy male material of the same age. On the ECG none of the patients developed signs of right ventricular impairment. A few patients had ST changes in the left precordial leads during work but were free from symptoms. The ECG findings ere not related to the degree of the scoliosis, the working capacity or the heart volume. The SD group had significantly lower working capacity than the LD group When related to previously obtained respiratory data the working capacity was significantly correlated to tidal volume and to vital and total lung capacities. In one third of the patients with low working capacity small circulatory dimensions were charac teristic feature. It could be assumed that restricted lung function would decrease the physical activity and hence the circulatory dimensions to an extent related to the degree of deformity

The cardiorespiratory failure in scoliosis has been widely documented, and for an extensive review reference is made to Bergofsky et al. (1). Most papers, however deal with clinical-pathological descriptions and thorough investigations of respiratory and hemodynamic function are missing. In the last 15 years a few reports have presented more elaborate cardiopulmonary data obtained through heart catheterization (1 9 10 16 19 25) but the origin of the skeletal deformity has mostly not been defined, the age of the patients has varied and the hemodynamic function has mostly been studied at rest. Actually only Bergofsky et al. (1) report catheterization findings at rest and during exercise in 6 patients, 19 to 63 years of age, with a diagnosis of idiopathic scoliosis. Consequently as regards the vital prognosis of idiopathic scoliotics and its relation to cardiopulmonary function, the data are rather sparse A hypermortality (15 17 18) and a predominance of heart and lung diseases as the cause of death ha e been reported. In the study of Nilsonne and Lundgren (17) a mortality ratio of 2.2 was observed in a group of patients whose diagnosis had been made 45–50 years previously As, to our knowledge, data from other follow-up periods of so long a duration have not been published, it was thought that an investigation of the pulmonary (6) and cardiovascular function within this group might add some data of alue in the consideration of therapy

A preliminary report was presented at the annual meeting the Swedish Medical Society in 1964.

MATERIAL

The study comprised 15 female patients ho had been diagnosed as idiopathic scoliotics at the Orthopedic Clinic at Karolinska Institutet about 50 years ago. The mean age at the examination of cardiovascular function was 63 years. The selection of the material is presented in previous study of their respiratory function (6). Vital characteristics are given in Table I. Two patients (nos. 2

Table I *Vital characteristics of 15 patients with scoliosis*

Case no	Age (y.)	Height (cm)	Weight (kg)	Degree of scoliosis (°)	Heart vol. (ml)	Blood vol. (l)	THb (g)	W_{max} sitting (kpm/min)
1	69	165	65	43	585	4.8	605	400
2	62	164	57	67	745	3.8	450	200
3	63	150	60	97	605	3.8	530	250
4	65	155	62	10	510	2.6	340	300
5	58	150	42	85	400	2.5	320	300
6	57	155	67	72	630	4.3	590	300
7	63	156	57	42	805	3.9	520	450
8	63	167	67	54	1045	4.4	620	400
9	65	147	52	86	720	3.3	435	300
10	64	163	65	15	560	4.2	695	300
11	57	135	42	102	565	2.2	270	200
12	63	150	62	80	830	4.2	535	200
13	65	159	64	13	—	3.4	430	300
14	67	162	67	48	850	4.6	725	350
15	63	153	63	84	425	4.1	575	250
Mean	63	155.4	59.5	59.9	663	3.7	509	300
S.D ±	3.4	8.43	8.26	30.6	179.3	0.79	134.0	75.6
Range	57–69	135–167	42–67	10–102	400–1045	2.2–4.8	270–725	200–450

and 5) refused cardiac catheterization. All the others completed the examinations as planned.

The material has been divided into two groups according to the severity of the deformation, i.e. one group of 7 patients with a mild to moderate deformity (LD group), the angle of the spinal curve being $< 60°$ (mean value ± SD $32 \pm 18.7°$), and one comprising 8 severely deformed patients (SD group) with an angle of $> 60°$ (mean ± SD $84 \pm 11.6°$). The LD group was on average years older ($p < 0.05$), 11 cm taller ($p < 0.01$) and 8 kg fatter ($p < 0.05$) than the SD group.

METHODS

Prior to the heart catheterization the subjects were thoroughly investigated. The methods employed for exercise testing, ECG recording, determination of oxygen consumption and for analysis of the blood samples are all commonly used in the Department of Clinical Physiology and have been described in a previous report of these scoliotic subjects (6). ECGs were recorded during three exercise tests, twice in sitting, once in supine position.

The *heart volume* was determined in the prone position by the method of Larsson and Kjellberg (14). The *total amount of hemoglobin* (THb) was determined by the alveolar CO method of Sjöstrand (21, 22). The *blood volume* was calculated from THb and the Hb concentration in finger blood. *Physical working capacity* (20, 22) was determined by tests in both supine and sitting position and is expressed as the working intensity at a heart rate of 170 (W_{170}) and 130 (W_{130}) or when a short extrapolation of the heart rate was impossible, only as W_{max}. In calculation of W_{170} no extrapolation of more than 28 beats was made.

Right heart catheterization was performed by the conventional technique via the left antecubital vein using cardiac catheters with single or double lumina. A teflon catheter was introduced into the right brachial artery by the percutaneous technique of Seldinger.

Blood pressures were recorded with an Elema differential transformer transducer EMT 490 A on an Elema "Mingograf" recorder. Mean pressures were obtained after electrical integration (time constant 0.8 sec). The reference point for zero pressure was taken at midthoracic level at the insertion of the fourth rib at the sternum.

Cardiac output was measured according to the direct Fick method. Blood samples were drawn during 1 min from the pulmonary and brachial arteries simultaneously with the collection of expired air. The oxygen content was calculated from the oxygen saturation and Hb concentration, using the factor 1.34 for the oxygen-combining power of Hb. A correction for physically dissolved oxygen was made according to Peters and van Slyke.

The *pulmonary resistance index* was calculated as the difference between the mean pressures in the pulmonary artery and the pulmonary arterial wedge position divided by the cardiac output.

Circulatory data were obtained both at rest in recumbent position and during supine exercise on an electrically braked bicycle ergometer.

The *degree of scoliosis* (Table I) has been estimated in terms of the angle formed by the converging limbs of the spinal curve, as described by Cobb (5).

Current statistical methods have been applied (23).

RESULTS

ECG findings

ECG at rest demonstrated in three patients ventricular or supraventricular ectopic beats on one of the recording occasions. There were no signs

of right ventricular strain or other pathological findings.

During exercise none of the patients developed ECG changes corresponding to the right ventricle. Normal ECG relations were found in seven of eight patients belonging to the SD group and in two patients with a moderate deformity One with an angle of 97 and three less de patients (10–43) had slight ST depressions in the left precordial leads during and after but were free from symptoms. The two remaining patients with angles of 13 (no. 13) 48 (no 14) developed signs of coronary insufficiency with pathologic ST depressions in the left precordial leads. They felt breathless but had no pains. The last patient was treated for a moderate systemic hypertension and had been taking digitalis for two years.

Working capacity

The W_{130} averaged 300 kpm/min (Table I) and was not affected by body position. Between the SD and LD groups there was a significant difference ($p < 0.01$) between the mean values (250 and 357 kpm/min) obtained in sitting position. In supine position corresponding data were 281 kpm/min and 321 kpm/min, but the difference was not statistically significant. The orthostatic heart rate reaction was similar in both groups.

The W_{170} in sitting position could be determined or calculated in six subjects of the LD group and in four of the SD group. The mean values, 567 and 488 kpm/min, were not significantly different. When related to THb the working capacity was lower than predicted in four patients. In relation to heart volume W_{170} was low in two of the same four subjects and in two others. The deviation from the predicted values was not directly correlated to the degree of deformity

THb and heart volum

Individual data are given in Table I and Fig 1 where the relation between THb and heart volume is illustrated THb and blood volume were both small in relation to body weight (22) and averaged 8.5 g/kg and 63 ml/kg, respectively They were not significantly influenced by the degree of scoliosis. Similar values have been observed in young wheel-chair patients (22) The

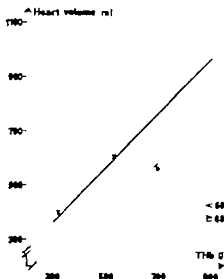


Fig. 1. Heart volume in supine position in relation to THb in 14 scoliotic subjects. Regression lines ±2 S D for healthy controls are given (3 11 13).

values of heart volume must be regarded as approximate due to the technical difficulties caused by the deformed anatomy However rough evaluation by inspection was indicative of cardiac enlargement in three subjects In case 8 belonging to the LD group, the value of 1 045 ml was approximate but the cardiac enlargement was general and moderate Catheterization data and ECGs at rest and during exercise were all normal. Case 7 had signs of a moderate and general cardiac enlargement but an ordinary hemodynamic function and normal ECGs. A moderate and general heart enlargement was also observed in case 9 who had normal ECGs, right entricular systolic pressures just below the upper normal limit at rest and during exercise (8) and history of scarlet fever in youth

Catheterization data obtained at rest and during exercise are presented in Tables II and III and Figs. 2–7

Oxygen consumption and mechanical efficiency (Table II)

Oxygen uptake at rest was 14 higher than the predicted basal value, i.e. comparable to similarly studied groups of non-fasting and non-hospitalized individuals. During exercise the mean oxygen consumption was 843 ml/min. Mechanical effi-

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9	65	147	52	86	720	3.3	435	300
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METHODS

Prior to the heart catheterization the subjects were thoroughly investigated. The methods employed for exercise testing, ECG recording, determination of oxygen consumption and for analysis of the blood samples are all commonly used in the Department of Clinical Physiology and have been described in a previous report of these scoliotic subjects (6). ECGs were recorded during three exercise tests: twice in sitting, once in supine position.

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Cardiac output was measured according to the direct Fick method. Blood samples were drawn during 1 min from the pulmonary and brachial arteries simultaneously with the collection of expired air. The oxygen content was calculated from the oxygen saturation and Hb concentration, using the factor 1.34 for the oxygen-combining power of Hb. A correction for physically dissolved oxygen was made according to Peters and van Slyke.

The *pulmonary resistance* index was calculated as the difference between the mean pressures in the pulmonary artery and the pulmonary arterial wedge position divided by the cardiac output.

Circulatory data were obtained both at rest in recumbent position and during supine exercise on an electrically braked bicycle ergometer.

The *degree of scoliosis* (Table I) has been estimated in terms of the angle formed by the converging limbs of the spinal curve, as described by Cobb (5).

Current statistical methods have been applied (23).

RESULTS

ECG findings

ECG at rest demonstrated in three patients ventricular or supraventricular ectopic beats on one of the recording occasions. There were no signs

Table III. *Pressures (mmHg) at rest and during supine exercise of 13 patients*

RV = right ventricle, PA = pulmonary artery PCV = pulmonary capillary vein, Bra = brachial artery S = systolic, diastolic, M = mean pressure, D = end diastolic

	RV		PA			PCV	Bra		
	S	D	S	D	M	M	S	D	M
Rest									
Mean	26	6	24	12	17	9[a]	142	77	103
S D	4 3	1.3	2 6	2.3	2 3	1.9	17 7	5 3	7.5
Range	19–34	4–9	21 28	9–17	14–21	6–12	120–177	67–86	91 114
Exercise (100–300 kpm/min)									
Mean	42	8	42	21	31	19[a]	175	89	122
S D.	6.7	2.2	7.0	3.8	4.9	5.8	18 8	7 0	16 5
Range	30–53	5–11	31 53	16–27	24–41	14–35	146–208	77 100	94–154

Number of patients – 12.

and during exercise are given in Table II. The relation between cardiac output and oxygen consumption is depicted in Fig. 2. At rest two patients had an increased cardiac output in relation to oxygen uptake, and during exercise another subject was slightly hyperkinetic; otherwise all values were within the normal variation. The cardiac output/l oxygen uptake was similar for the two groups of scoliotics both at rest (25 l) and during work (12 l)

Stroke volume

Individual data at rest and during exercise are given in Table II. The smallest volume, 35 ml, was determined at rest in the most severely deformed patient, case 11 The mean value at rest was 74 ml in the LD group and 61 ml in the SD group· during exercise corresponding values were 91 ml and 79 ml. Neither of the differences between the group means were statistically significant, nor was there a correlation between the degree of deformity and stroke volume On transition from rest to exercise the stroke volume increased on an average by 22% which is within the normal variation found in healthy old men (7) Figs. 3 and 4 present the relations of stroke volume during exercise to THb and to heart volume Within the scoliotic subjects there was no correlation between stroke volume during exercise and the two other circulatory variables.

Three of the individuals had a small stroke volume in relation to THb (Fig. 3) and one patient presented a borderline value. Two of the same patients had normal stroke volumes when related to heart volume (Fig. 4) and normal relations between heart volumes and THb (Fig. 1)

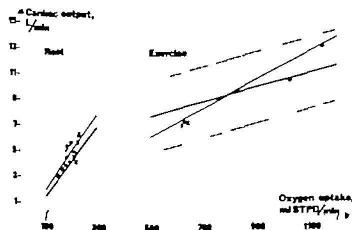


Fig. 2 Cardiac output in relation to oxygen uptake at rest (= 13) and during exercise (= 12) in scoliotic subjects ● = LD patients, = SD patients, = number of observations. Regression lines ±2 S.D. of healthy elderly males (heavy lines) (8) and regression lines (thin lines) of the scoliotics at rest and during supine exercise are presented.

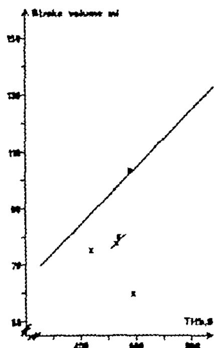


Fig 3 Stroke volume during supine exercise in relation to THb of 12 scoliotic subjects. Regression line ±2 S.D for healthy young subjects are given (2, 3 11 12) Symbols as in Fig. 2.

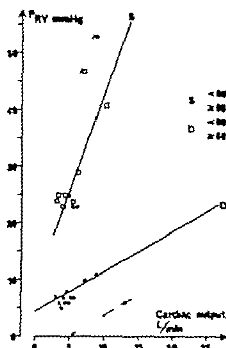


Fig 5 Right ventricular systolic (P_{S}, S) and diastolic (D) pressures at rest and during exercise in relation to cardiac output of 13 scoliotic subjects. Regression lines ±2 SD of healthy elderly males are given (8).

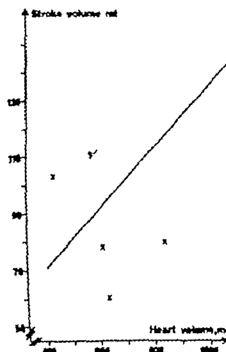


Fig. 4 Stroke volume during exercise in relation to heart volume in supine position of 11 scoliotics. Symbols as in Fig. 2. Regression line ± S.D for the same subjects as in Fig. 3.

The stroke volumes of the two remaining patients were small also when related to their heart volumes, but the relations were normal between the heart volumes and THb

Three of the other subjects with a small stroke volume in relation to heart volume had enlarged heart volumes in relation to their THb but normal ECG reactions during exercise In one case there was a history of scarlet fever (described under THb and heart volume).

Intracardiac and intravascular pressure

Mean values, ±S.D and the range of the individual data obtained at rest and during exercise are given in Table III. Individual right ventricular pulmonary arterial, pulmonary capillary venous and brachial arterial pressures in relation to cardiac output are depicted in Figs. 5–7

At rest case 11 the most severely deformed patient, had pulmonary arterial pressures at and above the upper normal limit. The pulmonary vascular resistance i.e the pressure drop over the pulmonary vessels in relation to cardiac output, was increased. Otherwise all pressures were normal and similar in both groups of scoliotics.

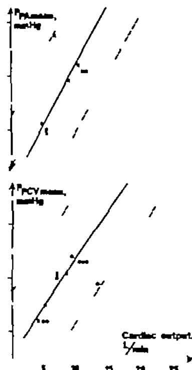


Fig. 6. Pulmonary arterial ($P_{PA\,mean}$) and pulmonary capillary venous ($P_{PCV\,mean}$) mean pressures at rest and during exercise in relation to cardiac output of 13 patients. Regression lines ±2 S.D. of the same elderly subjects as in Figs. 2 and 5 are indicated. Symbols as in Fig. 2.

During exercise all pressures increased ordinarily in relation to cardiac output (Figs. 5–7) when compared with the elderly male material (8) and with no difference between the two scoliotic groups. In the most severely deformed patient (no. 11) the exercise test during the catheterization had to be interrupted due to hyperpnea and arterial desaturation. The cardiac output determination could not be completed, but it could be reasonably assumed that the pressures in the pulmonary artery 53/ 7 indicated a pulmonary hypertension, which was present already at rest.

The pulmonary circulatory resistance index remained at the upper normal limit in SD subject 6. In case 4 no determination could be made. No statistical difference was established between the group means.

The brachial arterial pressures were all normal in relation to age, and increased similarly with increasing cardiac output in both scoliotic groups.

DISCUSSION

As has been considered in earlier papers (6 17), a positive selection has taken place which will somewhat reduce the alue of the present results for assessing cases of idiopathic scoliosis. Normal data for the corresponding age, sex and methods of investigation are not accessible which to some extent makes comparisons approximate. On the other hand hemodynamic studies of cases with

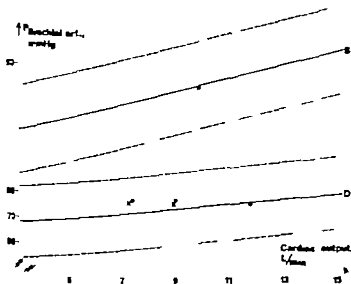


Fig. 7. Systolic (*S*) and diastolic (*D*) pressures in the brachial artery in relation to cardiac output during supine exercise in 12 scoliotics. Symbols and regression lines ±2 S.D. as in Figs. 2, 5 and 6.

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DRUG-INDUCED AGRANULOCYTOSIS

III Response to Corticosteroids

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bstract. After 3 days of corticosteroid treatment and ce ssation of the suspected drugs remission took place in en patients included in series of 20 patients with drug-nduced agranulocytosis. In four other patients spon- remissions were observed 5 to 7 days after cessa- of the suspected drugs. In three more patients the ming of the remission occurred after 6 to 9 days of ticosteroid treatment, thus not earlier than the spon- remissions. The result suggests that corticoster oids may speed up the recovery from agranulocytosis. favourable effect is probably limited to cases with an munological type of agranulocytosis.

There are varying opinions concerning the effect of corticosteroids on acute agranulocytosis. Some authors reject their value (12) while some support r use (11) perhaps limited to cases in which s remission does not occur within a week (5). An important reason for this may be variation of the pathogenetic mechanism lead- to this clinical condition (7 10) In addition overall prognosis of the disease is good (8) the effect of a particular specific treatment be evaluated by the frequency of remis- ons. Hence, we observed the relationship be- the beginning of the remission (1) and the of the causative drug (2) and the dura on of corticosteroid treatment to find out hether corticosteroids shorten the course of the sease.

PATIENTS AND METHODS

he series included 20 patients with acute agranulocytosis ring the period 1966–71 They all had clinical picture acute illness, sore throat, fever neutrophils less than al, and no evidence of other blood disorders in the pheral blood or bone marrow The causative drugs ac- cording to the histories are noted in Fig. 1

The patients were treated at the Department of Medicine, University of Oulu, at the Second Department of Medicine, University of Helsinki, and at five regional hospitals co-operating with Oulu. The treatment strictly avoided the use of all suspected causative drugs during antibiotic therapy to control infections. Corticosteroids were given at various intervals after cessation of the suspected drugs as indicated in Fig. 1 Most patients received prednisolone orally 30 to 60 mg daily or an equivalent amount of other corticosteroids. Patient 5 however received 40 units of ACTH daily i.m. and patient 6 received 200 mg of hydrocortisone i.m. on admission only White blood cell counts and their differential counts were performed daily The beginning of the remission, indicated by clear increase of neutrophils in the peripheral blood that continued during the following days, is presented by vertical arrow in Fig. 1

RESULTS

The figure indicates that the spontaneous remissions in four patients took place from 5 to 7 days after the cessation of the suspected causative drug. The response to corticosteroid therapy showed two patterns. a remission after 3 days in ten cases, and after 6 to 9 days in three cases. The time of the remission in the latter group coincides with that of the spontaneous remissions, indicating delayed or no effect of corticosteroids on the disease. Two out of three cases in the series with metamizol-induced agranulocytosis and one case caused by carbimazole belonged to this group. One of our carbimazole patients had a spontaneous remission the third had a remission on the 14th carbimazole-free day after 3 days of prednisolone treatment. This patient had received a high dose of carbimazole, 600 mg daily for 3 weeks, and the influence of corti-

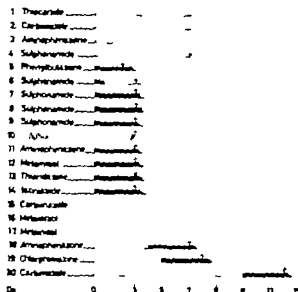


Fig 1 The course of agranulocytosis in 20 cases and the suspected causative drugs. Day 0 = the time of withdrawal of the suspected drug. ▮ = the period of corticosteroid treatment, † = the start of the remission.

costeroids on the course of the disease remains uncertain.

In ten patients the beginning of the remission occurred after a constant period of corticosteroid therapy i.e. 3 days. In five cases the causative drug was sulphonamide, and in one case each ... -one isoniazide, metamizol, phenylbutazone and thioridazine. The effect of corticosteroids remains uncertain in three cases treated later. Although their remissions occurred on the 4th day of prednisolone treatment, the withdrawal time of the suspected drugs was already as long or even longer than that required for the spontaneous remissions observed.

DISCUSSION

Our patients with agranulocytosis formed two groups according to the response to corticosteroid treatment. Ten developed a remission after 3 days of treatment and in three cases the time of remission fell within the range of the spontaneous remissions observed in four other cases. Two pathogenetic mechanisms have been described in drug-induced agranulocytosis: an immunologic (7) and a toxic (10). This may be the basic reason for the various responses to corticosteroids in different cases of agranulocytosis.

Corticosteroids increase the count of granulocytes in the peripheral blood (3). The initial phase of granulocytosis is apparently produced by a combination of an increased marrow release rate and decreased efflux of cells from the blood (2). After several days of corticosteroid treatment, both the marrow release rate and turnover time of granulocytes are normal (1) and the marrow granulocyte reserves are reduced (4). The plasma of neutropenic subjects has been found to increase the release of granulocytes from the marrow (6). Because the marrow release rate of granulocytes is increased and the granulocyte reserves of the marrow are exhausted in agranulocytosis, one cannot expect a further increase of the release rate of the granulocytes to be the mechanism underlying the favourable effect of corticosteroids in this condition.

In the immunological type of drug-induced agranulocytosis a shortened survival of the granulocytes in the peripheral blood, due to the action of antibodies, is the immediate cause of neutropenia (7). The spontaneous remission may be expected after the survival time of the granulocytes returns to normal after the antibody produced has been consumed, provided that no further antigenic stimulation of the antibody production occurs. In most of our cases of agranulocytosis treated with corticosteroids a remission took place after 3 days of treatment. The calculated delay of granulocyte response to infection in the absence of adequate marrow granulocyte reserves is 2 or 3 days (4). Hence it seems possible that, soon after the corticosteroid treatment began the destruction of granulocytes in these cases ended due to an inhibition of the action of antibodies.

The favourable effect of corticosteroids in agranulocytosis is probably limited to cases with an immunological type of the disease. Hence it is natural that the results with corticosteroids in agranulocytosis differ widely in different series of patients, depending on the distribution of the immunological and toxic cases. The fact that even the same drug may cause agranulocytosis by both of the known mechanisms (12) could explain the different response in our cases with metamizol as the suspected agent. However for this reason, the cases of agranulocytosis cannot be divided into immunological and toxic groups on the basis of drug history alone.

For the patient with agranulocytosis the deci-

ive treatment is strict avoidance of all suspected drugs during the disease (9) supported by an effective antibiotic therapy for infections. In addition, corticosteroids seem to shorten the course of agranulocytosis in most cases. In the remaining cases corticosteroids are neither effective nor harmful. For these reasons the use of corticosteroids is justified as additional treatment in ery case of agranulocytosis, until useful methods become available for finding out which cases ond to corticosteroid therapy.

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PRIMARY CARDIOMYOPATHY

A Prospective Clinical and Physiological Study

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Abstract. This prospective clinical and physiological study included ten consecutive patients with primary cardiomyopathy (obstructive and alcoholic cases excluded). Five of them were of the hypertrophic and five of the congestive type. The hypertrophic group was characterized by a systolic murmur of late onset and T wave inversion localized in the apical lead, while dyspnea, atrial fibrillation and cardiac enlargement were more common in the congestive group. In the exercise test the hypertrophic group as a whole performed the predicted work and the congestive group only half of it. During a follow-up period of 3–6 years two patients of each group died (sudden death). The two deceased patients of the congestive group were characterized by being the only two with persistent atrial fibrillation, having the lowest physical working capacity and being the only two in this group with abnormally high left ventricular end-diastolic pressure during exercise. In the hypertrophic group the two deceased patients were the only ones with dyspnea on effort and large heart volumes in relation to the total amount of hemoglobin. These two patients also had the lowest working capacity and the lowest end-diastolic pressure of the left ventricle during exercise in this group.

During the last decade numerous studies have been presented on the clinical, hemodynamic and angiographic characteristics of patients with primary cardiomyopathy. The majority of these reports are concerned with patients with obstruction of the outflow tract of the left ventricle (17, 22) while only a few deal with cardiomyopathy of the congestive type. It is evident from the recent paper by Goodwin (6) that the two manifestations of primary cardiomyopathy, the hypertrophic and congestive cases, differ to some extent in respect of clinical findings and some hemodynamic variables, such as residual fraction and end-diastolic volume of the left ventricle (4, 8), while they show similarities in other respects. It is also evident from the paper by Goodwin, *among others, that many patients of the hypertrophic type* do not have any pressure gradients within their outflow tracts.

Primary cardiomyopathy has been defined in different ways. Here it refers to a chronic myocardial disease which is not only primary but also idiopathic and non-obstructive. This definition excludes, among other cardiac diseases, also alcoholic cardiomyopathy and muscular sub-aortic stenosis.

The aim of the present communication was to further compare the clinical findings and the hemodynamics at rest and during exercise between patients with cardiomyopathy of the hypertrophic and the congestive type. Moreover it was considered worthwhile to re-examine the survivors some years later with regard to physical working *capacity, heart volume and tests of immunologic* diseases.

MATERIAL

During 1965–67 ten patients were diagnosed in the Department of Medicine as having cardiomyopathy which was primary, idiopathic and non-obstructive. During the same period two other patients were found to have muscular sub-aortic stenosis and several an alcoholic cardiomyopathy but these were not included in the study. Some anthropometric data of the ten patients, of whom four were men (age 22–54) and six women (age 23–50), are given in Table I.

The diagnosis of myocardial disease was based on a) dyspnea (when walking on even level or at rest) + ECG changes (left bundle branch block (LBBB), left ventricular hypertrophy (LVH), or T-inversion in lead CR_4) + pathological cardiac enlargement at chest X-ray: four cases (nos. 2, 6, 9, 10), b) dyspnea + ECG changes: two cases (nos. 3, 7), c) dyspnea + cardiac enlargement: one case (no. 8), d) ECG changes + cardiac enlargement: one case (no. 5), e) ECG changes (T-inversion in CR_4): two cases (nos. 1, 4).

Table I. *Some anthropometric, clinical and laboratory data on admission*

Case no.	Sex	Age (y.)	Height (cm)	Weight (kg)	Family history	First symptom at age	Symptoms at hospitalization	Rhythm, auscultatory and PCG *findings*
Hypertrophic group								
1	♂	22	180	63	–	ACP 18	ACP	SR. Normal 1st and 2nd sound. 3rd sound over 4th LICS. Ejection murmur with delayed onset grade 3, 3rd LICS
2	♀	31	164	57	–	DE 15	DE	SR. Normal sounds. Ejection murmur with delayed onset, grade 3 3rd LICS
3	♂	31	176	76	–	DE 29	DE	SR. Normal sounds Ejection murmur with delayed onset, grade 2, 3rd LICS
4	♀	21	172	60	+	PT 19	PT	SR. Loud 1st sound. Ord. 2nd sound. 4th sound, 4th LICS. Ejection murmur with delayed onset, grade 3, 4th LICS
5	♀	35	150	44	–	PT 31	PT	SR. Ord. 1st sound. Loud 2nd sound. 2nd LICS Ejection murmur with delayed onset, grade 3 4th LICS
Congestive group								
6	♀	44	162	66	–	AF 21	DE, LE	AF Ord. 1st sound. Loud 2nd sound. 2nd LICS. Ejection murmur grade 2, 3rd LICS
7	♀	35	162	64	–	DE 34	AE, LE, DR	SR. Normal sounds. Ejection murmur, grade 1 2, 4th LICS
8	♀	50	164	68	–	DE 44	DR	SR. Normal sounds. Ejection murmur, grade 2, 3rd LICS
9	♂	28	191	95	–	AF 28	DE, LE, R	SR. Normal sounds. No murmurs
10	♂	54	176	92	–	DE 35	AE, DR, LE	AF Loud 1st sound. Ordinary 2nd sound, 3rd sound apically Ejection murmur grade 2, apex

ACP = atypical chest pain, AE = angina on effort, AF = atrial fibrillation, D = dyspnea, DE = dyspnea on effort, [illegible]spnea at rest, F = fatigue, LBBB = appearance of left bundle branch block during exercise, LE = leg edema, LICS = left [illegible] space, PT = paroxysmal tachycardia, R = rales, SR = sinus rhythm

The interval between onset of symptoms and admission was 0–23, mean 8 years. For the four cases without cardiac enlargement as a sign of longstanding disease the interval was 1–4 years.

Coronary heart disease as the cause of the chronic myocardial disease was probably excluded because of the low ages at onset of symptoms, for men 18–35 and for women 18–42 years. Coronary heart disease is very rare in this country before the age of 35 for men and 45 for women (2). In no case was hypertension present at the first examination after onset of symptoms or at the examination on admission, and the fundi were normal. Valvular heart disease, intra- and extracardiac shunts and constrictive pericarditis were excluded by right and left heart catheterization and angiocardiography—see below. There was no support for diagnosis of cor pulmonale at physical examination, chest X-ray or spirometry.

The histories did not give any suspicion of chronic alcoholism, nor did they include any infectious illness or vaccination shortly before the onset of heart symptoms. In none of the women had the disease started during or shortly after pregnancy. The physical findings did not reveal any signs of neuro-muscular, collagen or amyloid disease. The laboratory examinations excluded thyroid and adrenal diseases. Serum sodium, potassium, magnesium and iron were normal. Rose's and LE tests and serum electrophoresis were also normal. Antistreptolysin titer, Paul Bunnell test, complement fixation test and dye test for toxoplasmosis were all negative. Chest X-rays, current and earlier, did not reveal any signs of sarcoidosis.

Thus a probable diagnosis of primary idiopathic chronic myocardial disease had been established in all ten cases. In three of them (nos. 4, 5, 6) the diagnosis was supported by a family history of cardiac death in early life. T o of these families have been presented earlier (1).

METHODS

The prospective part of the study *clinically* included detailed interview regarding conditions before the onset of symptoms that could have possible etiologic role

...me m²)	Angiocardiography	THb (g)	Work performance: Highest work load performed (kpm/min)	Min on highest load	Heart rate at the end of work (beats/min)	Symptoms when work was interrupted
	Hypertrophy of the upper part of ventricular septum	615	900	6	148	—
	Hypertrophy of the upper part of ventricular septum	490	400	6	14[illegible]	D, F
	Hypertrophy of the outflow tract of left ventricle	715	900	3	180	D A
	Hypertrophy of the upper part of ventricular septum	520	600	6	166	—
	Hypertrophy of the outflow tract of left ventricle. Mitral regurgitation	500	600	3	158	F
		640	400	5	170	D, A
		570	400	2	142	D, F A, LBBB
		560	400	6	125	D F
	Dilatation and symmetrical slight hypertrophy of the left ventricle	830	900	6	135	D
		720	150	6	90	D F

previous infectious diseases, travelling abroad, allergic symptoms, chest trauma, divergent food habits, and so on.

ECG at rest and during exercise were recorded with direct writing ink jet electrocardiograph (Mingograph 42 or 81, Elema-Schönander, Sweden). The following leads were used: I, II, III, VR, VL, VF and CR_1. During exercise the reference electrode was placed on the forehead (CH). *Phonocardiograms (PCG)* were recorded with the same Mingographs. *Physical working capacity* was determined in the sitting position on an electrically braked bicycle ergometer (Elema-Schönander) according to the protocol of Sjöstrand (20). The work was started at a load of 200 kpm/min for females and 300 for males, except in subject 10, who only worked at 150 kpm/min. The test was continued with progressive increments of 200 kpm/min for females and 300 for males in 6 min stages to a target heart rate of about 170 beats/min unless the subject before that was forced to stop the exercise due to subjective discomfort (dyspnea, fatigue, etc.). *Total amount of hemoglobin* (THb) was analyzed with the alveolar carbon monoxide method (19), and the blood volume was calculated from THb and Hb concentration. *Heart volume* was measured roentgenologically with the patients in both standing and prone position. The relative heart volume was calculated according to Jonsell (13) from exposures in standing position. Total heart volume was determined in prone position with exposures in two planes during quiet breathing (15). *Catheterization* of the right heart was performed from the left arm using a no. 8 Cournand catheter. The left ventricle was catheterized with a loop-end teflon catheter with an inner diameter of 1.2 mm inserted percutaneously in the right femoral artery. Blood pressures and cardiac output were measured in the supine position both at rest and during bicycle exercise. The load used was individually chosen from previous results, so that the patients were able to perform 10 min work without pronounced discomfort. *Blood pressures* were recorded with pressure transducers (EMT 31, Elema-Schönander) connected to Mingograph 81. The midthoracic line was used as reference level. *Cardiac output* was determined according to the Fick principle with blood oxygen saturation analyzed spectrophotometrically (12) and expired air analyzed with the micro-Scholander technique after collection of air in Douglas bags. *Angiocardiography* was performed at the Department of Radiology from the left and/or right ventricle in all cases with possibly or definitely pathological heart murmur or with an inverted T in the apical lead.

Table II. *ECG findings at rest*

	Case no.									
	Hypertrophic group					Congestive group				
	1	2	3	4	5	6	7	8	9	10
Rhythm										
Sinus rhythm	+	+	+	+	+		+	+	+	
Atrial fibrillation						+			+	+
Extrasystoles, ventricular		+			+	+			+	+
Mean QRS axis										
+105° – −30°	+	+	+	+		+	+	+	+	
< −30°					+					+
A V conduction										
Normal	+		+	+	+	+	+[b]	+	+	+
AV block							+[b]			
WPW		+								
Intraventricular conduction										
QRS-time ≤ 0.10 sec	+		+	+		+	+[b]	+	+	
Q > 0.04 in CR_4										+
LBBB		+					+[b]			
ALBBB					+					+
Hypertrophy										
Atrium			+	+	+				+	
Left ventricle			+	+		+				
S-T and T										
S-T depression > 0.1 mV[c]					+					
T-amplitude < −0.1 mV isolated or with max. in CR_4	+		+	+	+				+	

Occasionally [b] Interference between normal A V conduction and AV block I–II as well as between normal A V conduction and LBBB. Not associated with conduction disturbance, ventricular hypertrophy or digitalis treatment.

Calculations

Left ventricular work index (LVWI) (kg m/min/M²):
[(LV $_{mean}$ − LV$_{ed}$) · CI · 1.36]/1 000
CI = cardiac output/M²

Pressure time/min (PTM) (mmHg/sec/min): PTB · HR
PTB (pressure time/beat) = LV $_{mean}$ · SEP
SEP (systolic ejection period) sec/beat
HR (heart rate) beats/min

Follow-up

In late 1970 and early 1971 the patients were followed up. In the survivors the heart volume and work performance were determined in both groups, as well as the serum γ-globulins and antinuclear factor titers in the congestive group.

RESULTS

The angiographic examination showed hypertrophy of the upper part of the ventricular septum or of the outflow tract of the left ventricle in five cases (nos. 1–5). These patients, considered to belong to the group of hypertrophic cardiomyopathies according to Goodwin (6), will be reported separately regarding clinical and physiological results from the other five (nos. 6–10) forming the congestive group. Most of the data are collected in Tables I–IV.

Hypertrophic group

This group consisted of three women and two men, 21–35 years of age. Their cardiac symptoms, mainly dyspnea on effort and paroxysmal tachyarrhythmias, had started 2–16 (mean 6) years before admission, and these symptoms still dominated. Two subjects had a family history of sudden death, in one case at a relatively early age. In none of these relatives had asymmetrical hypertrophy of the heart been noticed at autopsy.

A constant *auscultatory and PCG finding* in this group was a systolic murmur of the crescendo–decrescendo type, with late onset. The interval between the first heart sound and the onset of the murmur varied between 0.03 and 0.05 sec. It was best heard along the lower left

sternal border (3rd–4th LICS) and toward the apex. *ECG* showed sinus rhythm in all patients; in two (nos. 2, 5) frequent ventricular extrasystoles were recorded. A left-deviated mean electrical axis (< −30) was present in one subject (no. 5). Two patients (nos. 3 4) showed signs of both atrial and left ventricular hypertrophy S-T depressions ≥0.1 mV without associated LBBB and LVH were recorded in one subject (no. 5) preferentially in lead CR_{4-7} In all cases negative T waves were recorded, which were associated with LVH in two cases (nos. 3 and 4) and LBBB in one case (no. 2). In four cases these T-inversions were isolated or maximal in the apical lead (nos. 1 3 4 5) In connection with the exercise test, ventricular extrasystoles appeared in two subjects (nos. 1 5) and S-T depressions developed or were accentuated in another two (nos. 2, 4). *Radiologically* one subject (no. 2) had a marked cardiac enlargement, and she also had by far the longest known history of all five patients. *Physical working capacity* was normal in *three patients (nos. 1 4 5). One woman* (no. 2) had to stop exercise at 400 kpm/min and a heart rate of 142 beats/min due to severe dyspnea and fatigue. One of the male subjects (no. 3) got dyspnea and chest pains after a few minutes' work at 900 kpm/min.

Hemodynamic findings. The mean pressure of the right atrium at rest exceeded the upper normal level (> 6 mmHg) in four patients (nos. 2 5). The *a* wave was of the giant type in two of these cases (nos. 3 4). The systolic pressure in the right ventricle was high (> 30 mmHg) in two subjects (nos. 4 5) and the right ventricular end-diastolic pressure abnormally elevated (> 6 mmHg) in three (nos. 1 4 5). The pulmonary artery wedge pressure was high (> 12 mmHg) in two patients (nos. 4 5). The systolic pressure of the left ventricle was normal in all cases and no pressure gradient was recorded in the outflow tract. The left ventricular end-diastolic pressure was abnormally elevated (> 12 mmHg) in four patients (nos. 1 2, 4 5) No pressure gradient was found between mean pressure recorded in pulmonary artery wedge position and the end-diastolic pressure of the left ventricle.

During exercise the systolic pressure of the right ventricle and the pulmonary artery increased in four subjects (nos. 1 2, 4 5) to values exceeding 50 mmHg. The filling pressure of the right ventricle increased further in two subjects (nos. 2, 4) while a considerable rise in the corresponding pressure of the left ventricle (> 20 mmHg) was recorded in all subjects.

Cardiac output at rest and during exercise was normal in relation to oxygen uptake in only one case (no. 1) and low in all others (Fig. 1) Stroke volume at rest was low (< 70 ml) in three subjects (nos. 3–5) and increased or was unchanged during exercise in all patients except one (no. 2) in whom it decreased from 77 to 59 ml. Pressure time/min was lower than normal in two patients (nos. 2, 3).

In two patients (nos. 3 4) hemodynamic measurements were performed before and after administration of digitalis (0.8 mg Cedilanid® Sandoz) In both cases left ventricular end-diastolic pressure was lower during exercise after digitalis. In the right ventricle the systolic pressure was decreased by digitalis in one subject (no. 4) both at rest and during exercise, while no effect was observed on the end-diastolic pressure *No intraventricular gradient was recorded following* digitalis. Cardiac output and stroke volume were increased by digitalis only in one subject (no. 3)

Congestive group

The congestive patients were in a higher age group (28–54 years, mean 42) than the hypertrophic. Admission to hospital occurred 0–23 years (mean 10) after the onset of symptoms. which were atrial fibrillation or dyspnea. One of the patients (no. 8) had had a diagnosis of rheumatic fever many years before the onset of the present symptoms. A second patient (no. 10) had, when 23 years of age, a short period of pain and swelling of knee and wrist joints in connection with high fever" thus possibly a rheumatic fever Two patients (nos. 8 9) had a goiter All subjects had a normal serum γ-globulin value on first admission. One of these congestive subjects (no. 6) had a *familial history* of cardiac death at an early age.

Four patients (nos. 6, 7 8, 10) had an early systolic murmur of grade 1–2 without delayed onset, best heard at the 3rd–4th LICS at the left sternal edge. No murmur was present in case 9 *ECG* showed atrial fibrillation consistently in two patients (nos. 6 10) and occasionally in one (no. 9) Ventricular extrasystoles were frequently

Table II. *ECG findings at rest*

	Case no.									
	Hypertrophic group					Congestive group				
	1	2	3	4	5	6	7	8	9	10
Rhythm										
Sinus rhythm	+	+	+	+	+		+	+	+	
Atrial fibrillation						+			+	+
Extrasystoles, ventricular		+			+	+			+	+
Mean QRS axis										
+105 – −30°	+	+	+	+		+	+	+	+	
< −30°					+					+
A V conduction										
Normal	+		+	+	+	+	+[b]	+	+	+
AV block							+[b]			
WPW		+								
Intraventricular conduction										
QRS-time < 0.10 sec	+		+	+		+	+[b]	+	+	
Q > 0.04 in CR_{4-7}										+
LBBB		+					+[b]			
ALBBB					+					+
Hypertrophy										
Atrium			+	+	+				+	
Left ventricle			+	+		+				
S-T and T										
S-T depression > 0.1 mV[c]										
T-amplitude < −0.1 mV isolated or with max. in CR_4	–		+	+	+				+	

[a] Occasionally. [b] Interference between normal A V conduction and AV block I–II as well as between normal A V conduction and LBBB. [c] Not associated with conduction disturbance, ventricular hypertrophy or digitalis treatment.

Calculations

Left ventricular work index (LVWI) (kg m/min/M²):

$[(LV_{mean} - LV_{ed}) \times CI \times 13.6]/1000$

CI = cardiac output/M²

Pressure time index (PTI) (mmHg, sec/min): PTB × HR

PTB (pressure time/beat) = LV_{mean} × SEP

SEP (systolic ejection period) sec/beat

HR (heart rate) beats/min

Follow-up

In late 1970 and early 1971 the patients were followed up. In the survivors the heart volume and work performance were determined in both groups, as well as the serum γ-globulin and antinuclear factor titers in the congestive group.

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Hypertrophic group

This group consisted of three women and two men, 21–35 years of age. Their cardiac symptoms, mainly dyspnea on effort and paroxysmal tachyarrhythmias, had started 2–16 (mean 6) years before admission, and these symptoms still dominated. Two subjects had a family history of sudden death, in one case at a relatively early age. In none of these relatives had asymmetrical hypertrophy of the heart been noticed at autopsy.

A constant *auscultatory and PCG finding* in this group was a systolic murmur of the crescendo–decrescendo type, with late onset. The interval between the first heart sound and the onset of the murmur varied between 0.03 and 0.05 sec. It was best heard along the lower left

ures (mmHg)

RV		PA			PCV	LV		Aorta			LVWI	PTM
S	eD	S	D	M	M	S	D	S	D	M	(kg m/min/M²)	(mmHg/sec/min)
29	7	28	12	16	12	110	14	118	78	98	4.4	2 100
55	7	52	20	31	24	153	40	154	94	110	11.3	4 300
25	6	23	8	13	12	98	14	100	57	71	2.4	1 700
53	9					128	20	123	80			
23	6	23	10	16	12	114	10	121	70	95	2.7	1 800
24	4	24	10	16		119	9	132	73	92		
35	8	38	19	25		118	25	134	81	95	3.2	2 900
36	7	31	18	23		131	18	139	81	103	5.0	2 500
37	11	36	20	24	19	128	30	126	81	96	3.2	2 600
31	9	27	13	21	15	130	29	123	82	107	4.1	2 700
60	14	62	29	40	32	139	40	136	92	106	4.8	3 500
48	14	42	21	31	29	140	30	132	95	114	4.6	3 900
31	12	25	12	18	13	142	14	136	73		2.9	2 000
51	12	44	25	32		155	26				7.0	3 700
33	5	28	12	17	14	194	15	194			4.9	3 500
36	9	56	28	38		200	23				11.8	5 300
29	9	30	11	15	8	117	12	128	76		3.8	2 000
31	12	31	21	23	16	123	17				4.0	2 300
32	10	28	12	18	12	135	13	136	75	103	5.6	2 700
40	14			21	17	160	15				7.5	3 800
19	6	17	12	15	14	116	10	115	91	108	2.8	2 200
47	11	45	29	39	27	145	15	135	93	110	7.2	3 700
26	5	25	11	14		152	33	152			3.0	2 700
29	10	29	14	20		175	45				4.9	3 700

Comparison between the hypertrophic and congestive groups

Dyspnea on effort was the main symptom of all the congestive patients, while only two out of five patients in the hypertrophic group complained of it. The dominating auscultatory finding in the hypertrophic group was a high frequency systolic ejection murmur with delayed onset. Four out of the five congestive patients also had systolic murmurs, which, however started immediately after the 1st heart sound and were more medium frequent. In both groups the systolic murmur was most pronounced at 3rd–4th LICS along the Certain differences in respect of ECG were found between the two groups. hus three patients belonging to the congestive had atrial fibrillation, while all in the ypertrophic group were in sinus rhythm. A T ersion, either isolated or maximal in the apical was recorded in four hypertrophic patients only one belonging to the congestive group. olume was markedly enlarged in four pa- out of five in the congestive group cor responding figures among the hypertrophic cases were one out of five. Physical working capacity was lower on the average in the congestive group than among the hypertrophic patients. Thus the congestive patients had to stop exercise at a much lower heart rate (mean 130 beats min) than the patients in the hypertrophic group (mean 162 beats/min) due to severe dyspnea and fatigue Right ventricular end-diastolic pressure increased during exercise in both groups to pathologically high alues. Also the left entricular end-diastolic pressure increased abnormally during work in subjects in the hypertrophic group, but in only two out of five congestive patients. No systematic difference was found in change of stroke olume during exercise in the two groups (Fig 2). When correlating the calculated left entricular work index to the left ventricular end-diastolic pressure at rest and during exercise, no differences were found between the two groups of patients (Fig. 3) Pressure time/min was low in three out of five hypertrophic patients, but only in one in the congestive group.

Table IV *Observations at the follow-up study*

Case no.	Alive	Dead	Time after onset of symptoms (y)	Time after first investigation (y)	Heart volume (ml/m²)	Rhythm	Work performance: Highest work load performed (kpm/min)	Min on highest load	Heart rate at the end of work (beats/min)	Symptoms bec. work was interrupted
Hypertrophic group										
1	+		10	5	340	SR	900	6	168	—
2		+	19	3						
3		+	5	2						
4	+		8	6	540	SR				
5	+		10	6	440	SR	600	3	125	D, F
Congestive group										
6		+	23	3/12						
7	+		7	5	470	SR	400	4	135	D, F
8	+		11	5	500	SR	600	4	143	D F
9	+		4	3	690	AF	900	6	165	
10		+	23	3						

Abbreviations, see Table I

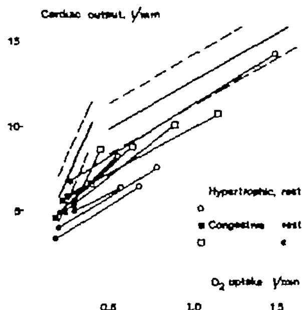


Fig. 1 Cardiac output in the supine position in relation to the oxygen uptake at rest and during exercise in hypertrophic and congestive cardiomyopathy. Heavy and dotted lines represent the regression lines ±1 standard error of estimate of normal individuals of comparable age group (14).

Follow-up study

At the follow-up in 1970–1971 (Table IV) two patients (nos. 2, 3) in the hypertrophic group had died 2 and 3 years after their first admission to Serafimerlasarettet and 5 and 19 years following the onset of symptoms, respectively. These patients were the only two in the hypertrophic group complaining of dyspnea on admission. In the congestive group two patients (nos. 6, 10) had died 3 months and 3 years, respectively after admission and in both cases 23 years after the onset of symptoms. These patients were the only two with atrial fibrillation on admission. The four patients who died during the follow-up period had the lowest physical working capacity in their respective groups.

The follow-up time for the three survivors in the hypertrophic group was 5–6 years from admission and 8–10 years from the onset of symptoms. In the congestive group 3–5 years had elapsed for the three survivors since admission and 4–11 years from the onset of symptoms.

All deaths were sudden. Two of the four deceased patients were autopsied, one in each group

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weight 730 g. Pericardium normal. Myocardium: hyper of the basal parts of both ventricles. Microscopy: ir cellular configuration with bizarre nuclear formation. Inflammatory cells. Endocardium, fibrotic performed

weight 800 g. Pericardium normal. Myocardium: dilata- l slight hypertrophy of both ventricles. Patchy fibrosis. al thrombi. Microscopy: moderate fatty infiltration. Inflam- ory cells

(nos. 2, 10) The autopsy confirmed the diagnosis did not give any clues to the etiology

Radiological heart volume was examined in all vors. It had increased significantly in three ents (nos. 4 7 9) and decreased in one no. 8)

Work performance was examined at the follow up in five of the six survivors. In no case had sical working capacity decreased during the interval of 3–6 years.

In all the three survivors in the congestive group, serum γ-globulin now had a borderline value. One of them (no. 8) had a positive anti-nuclear test, namely against smooth muscles in a titer of 1 10

DISCUSSION

The series presented in this paper has been divided according to Goodwin (6) into two main types—hypertrophic and congestive cardiomyo- This grouping was made by angiocardio- ry performed in all cases with possibly or initely pathological heart murmur or with a wave inversion maximal in the apical lead. Half

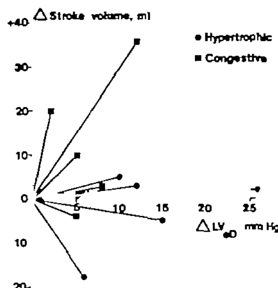


Fig. 2. Changes in stroke volume from rest to exercise in relation to corresponding changes in left ventricular end-diastolic pressure in hypertrophic and congestive cardiomyopathy

of the ten patients were found to belong to the hypertrophic and half to the congestive group in this consecutive series, from which alcoholic and obstructive cases had been excluded. One of the main purposes of this study was to see in what respects these two groups differed as regards clinical and hemodynamic findings, although we are fully aware that the groups are too small to give any conclusive information in these respects.

Dyspnea was a constant symptom in the congestive group. Only two of the hypertrophic cardiomyopathy patients complained of it, in fact the two who died during the follow-up period

A systolic murmur best heard at the left sternal edge and towards the apex, was present in all patients except one. In the hypertrophic group the murmur was characterized by a later onset, which agrees with the findings of others (1 6, 10) An insignificant right intraventricular gradient was recorded in one of these patients and no outflow obstruction at all in the others. Therefore as pointed out earlier by Goodwin (6), the murmur cannot be due merely to an outflow obstruction, but probably to a mitral insufficiency which in fact has been demonstrated in a high percentage of cases of hypertrophic cardiomyopathy In the present material, regurgitation to the left atrium was detected only in one patient.

Table IV *Observations at the follow-up study*

Case no.	Alive	Dead	Time after onset of symptoms (y.)	Time after first investigation (y)	Heart volume (ml/m²)	Rhythm	Work performance: Highest work load performed (kpm/min)	Min on highest load	Heart rate at the end of work (beats/min)	Symptoms when work was interrupted
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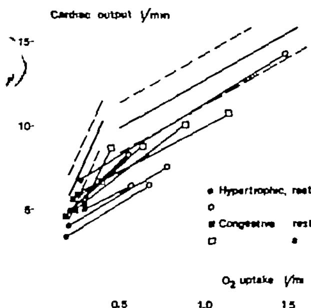


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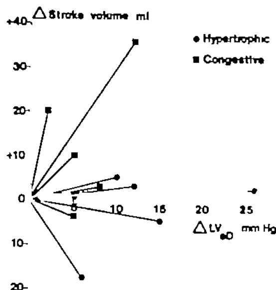


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FATTY ACID COMPOSITION OF SERUM CHOLESTEROL ESTERS, PHOSPHOLIPIDS AND TRIGLYCERIDES IN SERUM OF PATIENTS WITH PULMONARY INSUFFICIENCY

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ct. The concentration of cholesterol, triglycerides phospholipids in serum and the fatty acid composi- on of these lipid fractions have been examined in 11 pa- ents with respiratory insufficiency and the results com- l with those of 30 healthy subjects of the same age. al and esterified cholesterol concentrations were signif y lower in the patients than in the controls. No cant differences were observed in triglycerides and holipids. In each lipid fraction linoleic acid per- ages were significantly lower in the patients compared th the control group. The patients showed significantly igher percentages of oleic, palmitoleic and arachidonic id in the cholesterol esters and phospholipids than the ontrols. Other differences between the two groups were minor importance. The possible mechanisms for the riations in serum lipids in patients with respiratory sufficiency are discussed.

In cases of acute myocardial infarction, definite changes in serum lipid patterns with a decrease in total cholesterol have been described (13 18) Gas chromatographic studies of the fatty acid composition showed a decrease in linoleic acid percentages in cholesterol esters, triglycerides and phospholipid fatty acid (10) The exact mechanism responsible for the ariations has not yet been clarified. However it seems appropriate to consider whether or not similar changes in serum lipids can be elicited in other conditions of somatic stress. We hav therefore studied the fatty acid composition of cholesterol esters, triglycerides and phospholipids in patients with chronic pulmonary insufficiency and arterial hypoxemia.

MATERIAL AND METHODS

The study comprised 11 patients (10 men and 1 oman), aged 56 to 78 years, and control group of 30 healthy subjects of comparable age The patients were all suffering from pulmonary insufficiency due to chronic bronchitis, emphysema or tuberculosis of long standing. Blood sampling for lipid analysis was performed during an exacerbation of chronic insufficiency at the height of the failure, and usually within the first 24 hours after admission to hospital. The means and ranges of arterial blood gas values ere total-CO_2 (plasma) 32.8 (23.2–41.8) mEq l, pH 7 43 (7.30–7 48), pCO_2 50.1 (32–82) mmHg, HbO_2 77.9 (63.2–92.5) %

Venous blood for the lipid studies was draw in the morning following 1 -hour fast. After extraction of the serum the cholesterol esters, triglycerides and phospholipids were separated by column chromatography The lipid fractions ere hydrolysed and methylated according to the method of Stoffel et al. (16). Gas chromatography was carried out on Perkin-Elmer F 11 apparatus ith flame-ionization detector at column temperature of 195°C. The stationary phase of the column as chromosorb W 60–80 mesh, and the liquid phase 8% butanediol succinate polyester The methods and procedures used have been described in detail elsewhere (9)

Calculation of the fatty acid composition from the gas chromatographic patterns was made by multiplying relative retention time by peak height, both measured in mm on the recording paper The products for all fatty acids are added and the percentage calculated for individual fatty acids.

Total cholesterol and esterified cholesterol were deter mined by the method of Webster (17), and phospholipids by the method of Brun (2). Triglycerides were determined by modification of the method described by Laurell (11).

In all groups percentage values of fatty acids of less than 1% were regarded as trace amounts and are excluded from the Tables.

Statistical evaluations ere made by means of Student' t-test, p values higher than 0.05 ere considered to be insignificant.

RESULTS

Table I summarizes the concentrations of serum lipids for the lung patients and the control ma-

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Table I. *Serum lipids in patients with pulmonary insufficiency compared with controls*

Serum lipids (mg/100 ml)	Patients ($n=11$)		Controls ($n=30$)		Statistical evaluation
	$\bar{x}$	S.D.	$\bar{x}$	S.D.	
Total cholesterol	204.2	55.9	261.4	40.6	$p<0.01$
Esterified cholesterol	139.7	27.7	199.2	34.1	$p<0.001$
Phospholipids	240.3	57.7	262.1	33.2	p 0.05
Triglycerides	82.8	34.0	99.3	49.5	$p>0.05$

terial mean values and standard deviations (S.D.) in mg/100 ml are shown. This Table also records that the total cholesterol and esterified cholesterol levels of the patients are significantly lower than those of the control subjects.

The fatty acid composition in per cent of total fatty acids in cholesterol esters, triglycerides and phospholipids in sera from patients and controls is given in Table II. In all three lipid fractions—particularly in the cholesterol esters—the mean percentage of linoleic acid is lower in the patient group than in the healthy individuals. The decrease in linoleic acid is balanced by significantly higher concentrations of oleic, palmitoleic and arachidonic acid in the cholesterol esters and osphollpids.

DISCUSSION

Serum lipids in patients incapacitated by pulmonary insufficiency were found to differ from those of normal individuals. Not only was serum cholesterol generally lower but the fatty acid composition also varied. This was especially true of linoleic acid, for which the values in all lipid fractions were significantly lower than those for the controls. Patients with pulmonary insufficiency thus appear to experience the same disturbances in serum lipids as have previously been reported in the acute phase of myocardial infarction.

Several possible mechanisms may be responsible for the present findings. A low-fat and low cholesterol diet is known to bring about a decrease in cholesterol levels, and may also be the cause of the reduction in linoleic acid (12, 14, 15). Anorexia and nausea prior to hospitalization might have contributed to impaired consumption of food, including fat, although relevant anamnestic data to support this assumption are not available. However during short periods of low fat diet, triglycerides actually increase (7). In our study the low cholesterol level was combined with normal triglyceride values, indicating that factors other than a change in diet are probably involved. A decreased hepatic synthesis of the plasma lipoproteins—suggested as an explanation of the low level of cholesterol seen in a burn accident (1)—is not tenable for the present finding. The simplest explanation is that the particular serum lipid pattern observed is attributable to a change in the endocrine homeostasis secondary to the severe somatic condition with hypoxia. A decrease in cholesterol (3) as well as in triglycerides (5) has been observed after the administration of ACTH, indicating that the serum lipids are affected by several of the hormones involved in the stress reaction.

In the present study the response with regard to triglycerides was less well defined than that from serum cholesterol. It is possible that the other main lipoproteins do not react in the same way as cholesterol during exposure to stress. In

Table II. *Fatty acid composition of serum in patients with pulmonary insufficiency compared with controls*

Fatty acid	Patients (11)		Controls (30)		Statistical evaluation
	$\bar{x}$	S.D.	$\bar{x}$	S.D.	
Cholesterol esters					
Palmitic	11.2	1.3	10.4	1.0	p 0.05
Palmitoleic	4.6	0.9	3.8	0.9	$0.05>p>0.01$
Stearic	0.9	0.4	1.4	0.5	p 0.01
Oleic	23.6	2.9	20.0	3.3	p 0.01
Linoleic	44.3	1.7	[illegible]	6.0	$p<0.001$
Arachidonic	7.5	1.3	5.2	1.3	p 0.001
Phospholipids					
Palmitic	27.8	2.9	25.8	1.9	0.05 p 0.01
Palmitoleic	2.0	0.8	1.4	0.4	$p<0.001$
Stearic	13.4	0.8	13.7	1.2	p 0.05
Oleic	14.6	1.5	11.9	1.7	$p<0.001$
Linoleic	17.5	2.4	20.5	3.5	0.05 p 0.01
Arachidonic	8.8	1.4	6.9	1.4	$p<0.001$
Triglycerides					
Palmitic	24.8	4.1	22.8	2.7	p 0.05
Palmitoleic	5.4	1.3	6.1	1.4	$p<0.01$
Stearic	5.6	1.0	5.3	1.0	$p>0.05$
Oleic	42.3	5.0	40.4	4.0	$p>0.05$
Linoleic	10.7	1.6	14.1	4.1	$0.05>p>0.01$
Arachidonic	1.3	0.3	0.9	0.8	$p>0.05$

gram-negative septicemia a decrease in serum cholesterol has been reported when triglycerides and pre-β-lipoproteins were increased (6).

In view of the different relative levels of the precursor linoleic acid in the two groups, it was of particular interest to note that the relative amounts of arachidonic acid in the patients were higher than in the controls. Relatively high values of arachidonic acid may indicate that the synthesis is normal or increased in spite of low linoleic acid levels, and that the arachidonic acid content in serum is probably not a direct function of the amount of linoleic acid present. Similar findings have been reported in subjects subsisting on a diet deficient in linoleic acid (15), in patients with hyperthyreoidism (8) and in patients during the first week following acute myocardial infarction (10). It is possible that, regardless of the quantity of linoleic acid available, a limited amount is converted into arachidonic acid so as to maintain the level of this acid.

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EFFECT OF CHOLESTYRAMINE ON MINERAL EXCRETION IN MAN

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Abstract. Cholestyramine in the chloride phase (32 g/day) was given to four hyperlipidaemic patients under balance study conditions. The changes in blood chemistry were slight, but urine pH decreased and the urinary excretion of chloride increased by about 70 mEq/day corresponding to about 60% of the chloride content of the resin. Urinary excretion of calcium increased significantly (1.–60%) in every subject, while faecal excretion did not show consistent change. Slight equidirectional changes in the excretion of magnesium were observed in every patient, whereas the change in phosphate excretion was negligible. Continued administration of cholestyramine with reduction of the total chloride load to pretreatment values essentially abolished the effect on calcium excretion. Ammonium chloride produced comparable changes in the excretion of calcium. Urinary citrate was followed in one patient and was strongly depressed during the period when chloride excretion was increased. Only transient changes in urinary sodium and potassium were observed. In one patient observed after two months treatment with cholestyramine the effect on calcium excretion was still apparent. It is concluded that cholestyramine affects mineral balance mainly through its chloride content and it is suggested that care should be taken to decrease the excess chloride load during cholestyramine treatment in order to avoid the development or progression of osteoporosis in patients at risk in this respect.

Cholestyramine, an anionic resin which binds bile acids in the bowel and thus increases their faecal excretion, is used in long-term treatment of several diseases, e.g. familial hypercholesterolaemia (8, 9), biliary cirrhosis (4, 15) and steroid-wasting enteropathy (16). Severe side-effects are rarely seen but there has been one report of non-respiratory acidosis in a patient with enteropathy (16) and others of right upper quadrant calcification in patients with biliary cirrhosis (15, 18). Constipation and mild steatorrhoea (7) may be induced, but compensation for the caloric loss is readily achieved by increasing the caloric intake. The effect on the nitrogen balance is negligible (9). Reports concerning the effect on calcium absorption are conflicting. Briscoe and Ragen (3) observed an increase whereas other investigators have reported no effect of cholestyramine on calcium absorption (17). Cholestyramine, on the other hand, was observed to impair the absorption of vitamin D_3 (17).

No data are available on the effect of this or related anion resins on the mineral balance and it seemed essential to investigate this problem in view of the fact that these drugs are given for years both to young and elderly people with varying degrees of more or less physiological osteoporosis. Even minor disturbances in mineral metabolism may thus be deleterious when their effect is cumulative over several years.

MATERIAL AND METHODS

The study was carried out on three patients with familial hypercholesterolaemia (type II) and on one hypertriglyceridaemic patient (no. 3 in Table I) who, in addition, had coronary disease, gouty arthritis and hypercalciuria but otherwise normal renal function. The hypercalciuria may have been due to hyperuricaemic nephropathy but was more probably an independent phenomenon. Ammonium chloride loading and tests for urine concentrating capacity were normal in all four patients. Two patients (nos. 1 and 4 in Table I) had no other metabolic disturbance. Patient 2 had coronary disease and mild diabetes mellitus. None of these three patients had any signs of disturbed renal function or mineral metabolism as judged by routine clinical, X-ray and laboratory investigations.

The patients were admitted to a metabolic ward and put on an isocaloric diet containing constant amounts of calcium, magnesium, sodium, chloride and phosphate as indicated in Table I. The diet was low in calcium and sodium chloride. Calcium as glucono-lactobionate (Calcium-Sandoz®) and sodium chloride in weighed amounts were added to provide the planned total intake. Ammonium chloride was added in gelatin capsules as

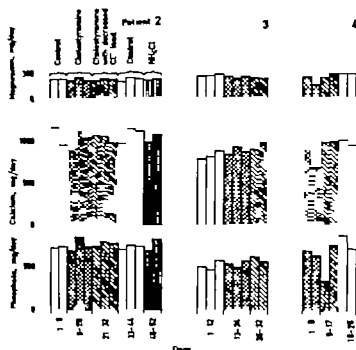


Fig. 2. Effect of cholestyramine (Cuemid®), cholestyramine with decreased chloride load and ammonium chloride on the daily faecal excretion of magnesium, calcium and phosphate in patients 2–4

shown in Table II. As expected, the drug decreased serum cholesterol and augmented faecal bile acid excretion but had no consistent effect on faecal fat or neutral sterol output.

In patient 1 (Fig. 1) 32 g of cholestyramine/day increased urinary chloride excretion from about 90 to a new steady state level of 140 mEq/day. Urinary calcium excretion increased simultaneously from 170 to an average of 240 mg/day while the output of phosphate and magnesium remained essentially unchanged. When the drug was discontinued, the excretion rates tended to return to the pretreatment levels.

In patient 2 cholestyramine had a stronger effect on chloride excretion, raising it from 90 to about 175 mEq/day. The pH of the urine fell from 5.8 to 5.2 and the excretion of calcium increased from 170 to about 270 mg/day. The urinary excretion of magnesium rose slightly but significantly ($p < 0.01$) while that of phosphate decreased slightly ($p < 0.01$). To compensate for the chloride contained in the resin, 75 mEq sodium bicarbonate was substituted for sodium chloride, while the resin treatment was continued at a constant dose. Consequently the urinary excretion of chloride, calcium, and magnesium decreased, while urine pH returned to the control value and phosphate remained depressed. When cholestyramine was discontinued and normal electrolyte intake was resumed, all the mineral excretion rates returned to the pretreatment levels.

In order to assess the effect of an isolated chloride load, 75 mEq of ammonium chloride in gelatin capsules was administered during the last period. The excretion of chloride, calcium and magnesium increased, as they did during cholestyramine medication. The excretion of phosphate, however, did not decrease as during administration of cholestyramine chloride. These experiments suggest that the effect of cholestyramine on the urinary output of magnesium and calcium is accounted for by its chloride content.

Patient 3 had an average daily excretion of 340 mg of calcium in the urine (Fig. 1) and about 800 mg in the faeces (Fig. 2) during the control period. Cholestyramine further increased urinary calcium but only to a small extent (average 380 mg/day) while chloride excretion increased by about 75 mEq, as in the normocalciuric patients. The output of magnesium was significantly augmented, while that of phosphate remained unchanged. Reduction of chloride intake

Table II. *Effect of cholestyramine (resin) on serum cholesterol and faecal fat and steroids in four hyperlipidaemic patients (mean ± S.E.)*

Treatment	Serum cholesterol (mg/100 ml)	Faecal lipids (g/24 h) Bile acids	Neutral steroids	Total steroids	Fat
None	343 ± 77	0.187 ± 0.039	0.712 ± 0.138	0.898 ± 0.127	2.65 ± 0.93
Resin	272 ± 26	1.482 ± 0.182	0.681 ± 0.179	2.161 ± 0.291	3.75 ± 1.51
Change	−71 ± 15	+1.295 ± 0.146	−0.031 ± 0.078	+1.264 ± 0.220	1.10 ± 0.67

Statistically significant changes.

lowered the calcium output and increased urine pH to the pretreatment levels, while no significant change was seen in magnesium or phosphate.

In order to study the effect of more prolonged administration of cholestyramine the drug was given to patient 4 for 2 months before admission to the metabolic ward. Upon admission the standard diet was started and cholestyramine treatment was continued. When urinary calcium excretion had stabilized, urine and faeces where collected in the usual way for 8 days. Sodium bicarbonate (75 mEq) was thereafter substituted for sodium chloride during 9 days, after which the resin was discontinued and the standard diet resumed.

It is seen from Fig. 1 that, as in case 2, the uction in the total chloride load immediately ecreased the urinary excretion of calcium which remained at the same level when cholestyramine was discontinued and a normal intake of chloride was resumed. It thus seems probable that the effect of cholestyramine on urinary excretion of calcium persists for at least 2 months.

Though cholestyramine markedly increased the faecal output of bile acids (Table II) no significant change was found in the faecal excretion of minerals (Fig. 2). Nor was the total mineral balance altered consistently.

Cholestyramine had no significant effect on blood pH in patients 1, 2 and 3, though in the latter patient the base excess varied between −1.8 and −.1 mEq/l off and between −2.5 and −4.0 mEq/l on cholestyramine indicating a slight, compensated acidosis during the treatment. In patient 4 the capillary blood pH was 7.36–7.37 after 2 months on cholestyramine and increased to 7.40 during the subsequent control period. At the same time the base excess increased from values between −3.2 and −.0 mEq/l during medication to +1.0 mEq/l during the control period. Urine pH increased simultaneously (Fig. 1).

The mean change in serum chloride concentration caused by cholestyramine or changes in electrolyte intake or both, was less than 1 mEq/l in all the patients. Thus the chloride load caused only small changes in the extracellular fluid electrolytes, whereas the changes in urine electrolytes were more pronounced. Urinary citrate, which is thought to reflect the intracellular pH (11), was determined in patient 4 and amounted to 1.16 ± 0.13 mmol/24 h during the last few days of cholestyramine administration. When sodium bicarbonate was substituted for sodium chloride citrate excretion increased to 3.29 ± 0.68 mmol/24 h and increased further to 4.00 ± 0.23 mmol/24 h during the subsequent control period. The prolonged chloride load may thus have induced marked intracellular changes.

The sodium and potassium balance of the patients did not change significantly except for transient increases in the amounts excreted in the urine during the first few days of each chloride load and reciprocal changes after the termination of excess chloride administration. The net changes were small and in the new steady state the balance was neutral in every instance.

DISCUSSION

Cholestyramine in the chloride phase markedly and consistently increases the urinary excretion of calcium and to a smaller extent that of magnesium (Table III). This effect persists for at least 2 months, i.e. the longest period of observation employed in the present study. Ap-

Table III. *Effect of cholestyramine (resin) and cholestyramine without excess chloride (resin – Cl) on the daily renal excretion of electrolytes (mean and range)*

Treatment	Chloride (mEq/24 h)	Calcium (mg/24 h)	Magnesium (mg/24 h)	Phosphate (mg/24 h)
None (pats. 1–4)	107 (89–124)	201 (83–349)	129 (96–187)	982 (627–1 465)
Resin (pats. 1–4)	177 (140–194)	256 (121–386)	148 (104–236)	942 (583–1 341)
Change	+70 (+41–+89)	+55 (+37–+96)	+19 (–2–+49)	–40 (–103–+76)
Resin – Cl (Pats. 2–4)	115 (95–128)	200 (81–319)	160 (109–234)	930 (785–1 265)
Change	+5 (±0–+10)	–2 (–30–+25)	+20 (+6–+47)	–170 (–230––82)

parently it is due to the increased absorption of chloride from the gut, because reduction of total chloride intake reduced the calcium output significantly (Table III). The daily dose of cholestyramine contained about 112 mEq chloride and the observed increase in urinary excretion varied between 50 and 90 mEq, i.e. about 45 to 80 % of the amount administered. The absorbed chloride was probably mainly exchanged for bicarbonate (5), the result being an increased acid load. In all the patients studied this was associated with marked changes in the urinary electrolyte output, whereas only slight changes occurred in the extracellular electrolytes. However, Stanley has reported a case of marked non-respiratory acidosis resulting from cholestyramine treatment in a patient with enteropathy (16).

The calciuretic effect of an acid load is well known, and ammonium chloride has been shown to produce experimental osteoporosis in mice fed on a calcium-depleted diet, whereas bicarbonate protects against osteoporosis under these circumstances (1). In view of the suggested role of the kidney in preserving the body stores of calcium (12), partial substitution of a bicarbonate-yielding anion for chloride would appear advisable in patients undergoing cholestyramin treatment. If this is not done, one could administer sodium bicarbonate routinely along with cholestyramine in the chloride phase. The former would, according to the present investigation, abolish the main effect of cholestyramine on urinary calcium excretion. The effect of bicarbonat was not specifically investigated but would in all probability reduce cholestyramine induced calciuria. However, it would result in a considerably increased intake of sodium, which in itself has a calciuretic effect and may also be undesirable for other reasons, for example in patients with cardiovascular disease. Therefore, reduced intake of chloride seems advisable during long-term cholestyramine treatment, particularly if osteoporosis is already present or may be expected to develop for any other reason.

It should be noted that there was no significant change in the faecal excretion of calcium or phosphate. The absorption of these minerals was thus not impaired, but on the other hand there was no decrease in the faecal excretion to compensate for the increased urinary excretion. The fact that the net balance did not change significantly may well be due to the short period of observation. Reports concerning the effect of cholestyramine on calcium absorption are conflicting (3, 17) and further research appears necessary to settle this point. Since the absorption of calcium from the gut is well known to be increased by an acid environment in the intestine, the effect of cholestyramine on calcium absorption may partly be pH-dependent. It would therefore be interesting to know the effect of cholestyramine on intestinal pH in the different absorption studies reported.

Although urinary calcium increased as a result of cholestyramine administration, there was probably no increase in the activity product of brushite in the urine. This product is considered critical for the formation of renal calculi (13). In this respect the effect of increased calcium activity was probably more than offset by the simultaneous decrease in urine pH, which, on the other hand, may favour development of urate stones. Urinary phosphate did not increase in any of the patients receiving a 12-day course of cholestyramin, and it decreased significantly in one of them. During the administration of ammonium chloride, phosphate excretion was significantly higher than during cholestyramine medication.

while the increase in urinary chloride was somewhat smaller (patient 7). However, in patient 4 who received a longer course of treatment with cholestyramine, urinary excretion of phosphate probably decreased when the drug was discontinued, although the decrease did not reach statistical significance. The effect of cholestyramine on urinary phosphate excretion may thus be time-dependent, whereas the effect on calcium excretion appeared to remain unchanged during the experimental period.

ACKNOWLEDGEMENT

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SPECIFIC DETERMINATIONS OF PROTHROMBIN DURING ANTICOAGULANT TREATMENT

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Abstract. A specific two-stage prothrombin assay has been applied for the control of oral anticoagulant treatment in comparison with one-stage prothrombin assay and an immunological assay of reactive prothrombin, as well as with Thrombotest and the prothrombin-proconvertin (P-P) method and with specific assays of factors IX and X. All the methods used were fairly well correlated but showed different activity levels. Prothrombin values of 20–45% corresponded to P-P values of 10–35% and Thrombotest values of 5–20%. Values of the present two-stage prothrombin assay were in accordance with prothrombin values immunologically determined. A large number of patients on long term anticoagulant treatment were routinely controlled with Thrombotest, patients with complications with the present prothrombin method as well. Minor bleeding complications occurred in patients with values within the therapeutic range of both methods and to the same extent. The activity of the inhibitor induced by the treatment was about the same in patients with bleeding complications as in the other patients. It does not seem necessary for the treatment to register this activity. The specific prothrombin assay seems to be as useful an indicator of the effect of anticoagulant treatment as the methods generally used.

For the control of treatment with dicumarol and related compounds there are several methods available. Thrombotest (18) the prothrombin-proconvertin (P-P) method according to Owren and Aas (20) and a modification of this method with Simplastin A reagent (9) are the methods mostly used in Scandinavia. These methods register factors II (prothrombin) VII and X, and Thrombotest in some respects also factor IX. This report concerns the application of a specific prothrombin assay for the control of oral anticoagulant treatment.

METHODS

Prothrombin was determined, 1) by the two-stage method previously described (14), 2) in some samples (23) by one-stage method according to Koller et al. (8, 3) in some samples ($n = 14$) by Ganrot and Niléhn at the Department of Clinical Chemistry Allmänna Sjukhuset, Malmö, Sweden, with their immunological method (4) based on antigen-antibody crossed electrophoresis (10). The normally reacting portion of prothrombin was determined and expressed in % of the total amount of prothrombin (5).

P-P was determined according to Owren and Aas (20).

Thrombotest (18) was performed according to the directions of the producer (Nyegaard, Oslo, Norway).

Factor IX was determined according to Nilsson et al. (13).

Factor X was assayed according to Bachmann et al. (1) with commercial reagents (3) from Diagnostic Reagents, Thame, Oxon, England.

The methods were standardized as described elsewhere (15). When capillary blood was used, the results were not corrected for hematocrit variations, which were not considered to influence the results significantly in the material presented.

MATERIAL

Three groups of patients from Karolinska Sjukhuset, Stockholm, were investigated during treatment with oral anticoagulants.

I. Fifteen patients, aged 30–65 years, were treated with dicumarol by Dr R. Cronestrand at the Thoracic Department, Karolinska Sjukhuset, in connection with surgery for occlusive arterial disease. The plasma samples were collected preoperatively and on the 3rd and 7th postoperative days. The treatment was started days after the operation.

II. Eight patients, aged 29–69 years, were referred to the Coagulation Laboratory Karolinska Sjukhuset, on account of difficult adjustment to proper level of anticoagulant treatment. Five patients suffered from recurrent episodes of thrombosis, one from intracranial thrombosis, two were treated postoperatively because of earlier episodes of thrombosis and pulmonary embolism.

III. Patients controlled at the outpatient clinic for anticoagulant treatment of the Coagulation Laboratory Karolinska Sjukhuset, altogether 615 mainly patients with coronary disease and occlusive arterial disease.

Table I *Prothrombin in patients before and during postoperative treatment with dicumarol*

Values in % of normal plasma activity
A = preoperative sample, B = 3rd postoperative day C = 7th postoperati day

	Prothrombin						
Pat. no	Two-stage method	One-stage method	Immunological method	P P	TT	F IX	F X
1 A	129	104	95	98	46	81	91
B	63	62	50	23	12	49	30
C	26	25	27	11	6	16	8
2 A	118	121	94	99	47	124	87
C	48	45	45	43	16	100	31
3 A	129	121	107	128	54	99	165
B	54	58	53	20	14	40	33
C	28	24	23	14	8	20	10
4 A	120	121	86	111	56	86	89
B	85	86	61	53	34	67	60
C	36	35	28	18	10	21	16
5 A	140	145	99	107	52	212	98
B	58	71	43	22	13	83	25
C	33	37	25	20	9	33	15

RESULTS

In the five patients with occlusive arterial disease, prothrombin was determined by the one-stage method of Koller et al. (8) and by the specific two-stage method earlier described (14) as well as immunologically (5) Moreover Thrombotest assays and determinations of P P factors IX and X were performed.

On the average Thrombotest showed the lowest [illegible], factor X values were somewhat higher P intermediate and prothrombin and factor IX values were the highest. The prothrombin values obtained with the one and two-stage methods were similar (Table I) The immunological method showed somewhat lower values, but was not standardized against the same normal plasma.

The eight patients referred to the Coagulation Laboratory were controlled with the two-stage prothrombin assay and Thrombotest during long-term treatment with dicumarol (6 patients) Marcoumar® (1 patient) or Waran® (1 patient). Factors IX and X as well as P P were determined during the first 6–7 weeks when the patients were treated by us and in three of the patients also after one year (Table II) Results from two patients are shown in Figs. 1 and [illegible]. The coagulation factor activities registered seemed to be similarly influenced by the treatment, but on

Table II *Prothrombin and related factors during long-term anticoagulant treatment*

Values i % of normal plasma activity

				Prothrombin					
Pat. no.	Sex	Age (y)	Date	T o-stage method	One-stage method	P P	TT	F IX	F X
1	♂	60	12.3	46	55	64	28	61	28
			19.3	48	50	65	26	48	36
			9 4	22	26	23	9	39	13
			16 4	26	24	30	13	41	14
2	♀	58	12 3	46	50	43	16	46	32
			19.3	54	45	44	11	32	32
			2.4	31	35	25	8	35	17
3	♂	69	2.4	37	48	48	14	78	[illegible]
			16.4	46	46	50	15	63	[illegible]

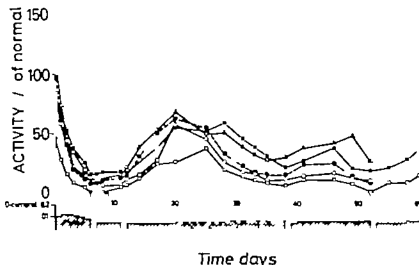


Fig. 1 Women, aged 30 years, treated with dicumarol on account of recurrent thromboses. Dicumarol dosage g day. ■ Prothrombin; △, factor IX, ● P-P; ○ factor X, □, Thrombotest.

different activity levels, as described for the other group.

In the patients studied after one year of treatment (Table II) the relations between the different coagulation factor activities agreed with those found in the initial stage of the treatment (Table I) except for the P-P values, which were higher in relation to the other assays after one year than initially. As reagents were produced and standardized in the same way for both investigations, the difference observed would probably not be due to methodological variations, but to increased proconvertin (factor VII) activity during steady-state treatment (19).

In the patient groups described above (Tables I and II) prothrombin values (14) were well correlated to Thrombotest values (= 0.94) to factor X values ($r = 0.92$) to P-P values ($r = 0.89$) and showed a fair correlation to factor IX values ($r = 0.77$). Thrombotest values were well correlated to factor X values ($r = 0.91$).

The relation of prothrombin values (14) to Thrombotest and P-P values in the patient groups described above is illustrated in Figs. 3 and 4. As can be seen, prothrombin values of 20–45 corresponded to Thrombotest values of 5–20% and P-P values of about 10–25%. Calculated with the clotting-time ratio method of Biggs and Denson (2) in a limited number of samples ($n = 22$) prothrombin 20–45% corresponded to Thrombotest 6–12%.

No major bleeding complication was observed

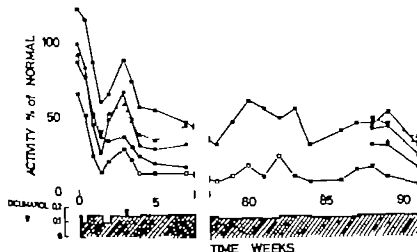


Fig. 2 Women, aged 48 years, treated with dicumarol on account of recurrent thromboses. Dicumarol dosage g day. □, Prothrombin (one-stage). Other symbols as in Fig. 1.

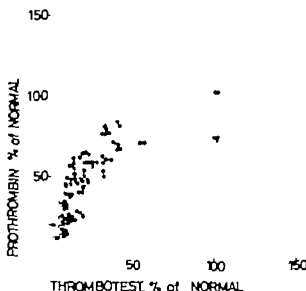


Fig 3 Correlation of prothrombin and Thrombotest values in patients on oral anticoagulant treatment.

in patients with prothrombin above 20% and Thrombotest above 5%. One patient who was treated with estrogen hormones for a prostatic carcinoma developed a venous thrombosis at levels within the thrombo-prophylactic range (prothrombin 41% Thrombotest 11%)

In the patient group III ($n = 615$) most patients were treated with dicumarol and a few with Waran[R]. The treatment was routinely controlled Thrombotest in patients with complications prothrombin determinations were performed in addition. In 70% of the 1 200 investigated samples Thrombotest was 5–15%. In 4% of the samples < 5%. No thrombotic episode was observed in this group. Ten of the 615 patients had mild bleeding symptoms but no severe bleeding complications occurred. Seven patients were treated for occlusive arterial disease, one on account of frequent thromboses. Two patients were treated postoperatively. Prothrombin and Thrombotest values were obtained simultaneously during a bleeding episode (Table III). In the patients with bleedings prothrombin values were 7–43% and Thrombotest values < 5 11% i.e. several of the patients had values within the therapeutic range: 6 of 10 patients with the specific prothrombin method, 7 of 10 patients with Thrombotest. Prothrombin 20–45% and Thrombotest 7–15% (9 11) were considered as therapeutic ranges. However several of these patients had medication or complicating disorders that could cause an increased bleeding tendency

It has been shown that an endogenous inhibitor of prothrombin activation (PIVKA) occurs during treatment with coumarin congeners (7). The discrepancy between values obtained with different control methods is considered to be due to varying sensitivity to this inhibitor (9 11). The inhibitor was determined graphically according to Hemker et al (7) in 12 patients with Thrombotest values within the therapeutic range in 6 patients with and in 6 without bleeding complications. The values obtained were similarly distributed in the two groups. The mean value of the patients with bleedings was somewhat lower than that of the other group, but the difference was not statistically significant.

In occasional patients the ratio prothrombin/Thrombotest values was lower than in the other patients, i.e. 1.5 1 to 2 1 instead of 3 1 to 5 1 (patient 1 Table II). These patients required a comparatively low dosage of the coumarin derivative used. The low ratio could not be due to depression of prothrombin values by increased antithrombin, as variations in antithrombin did not influence the assay significantly (15). However the activity of the inhibitor (7) determined in one of these patients was lower than mean − 2 S.D of 10 other patients with a prothrombin/Thrombotest ratio 3 1 to 5 1 (Fig. 5)

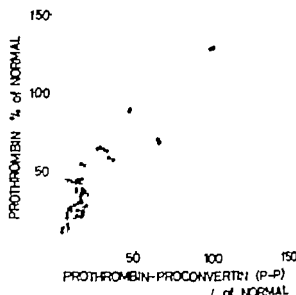


Fig 4 Correlation of prothrombin and P-P values in patients on oral anticoagulant treatment.

DISCUSSION

Methods which register several of the vitamin K dependent coagulation factors (factors II, VII, IX and X) have been considered more suitable for the control of treatment with coumarin derivatives than specific assays of prothrombin (11 19 21 22). When the synthesis is blocked, factor VII is de creased most rapidly followed by factors IX and X, and lastly prothrombin depending on the dif ferent half-life of the factors (6, 11) Conse quently there might be a risk of low activities of factors VII, IX and X in patients in whom the prothrombin level has not yet decreased.

The aim of the present investigation was to compare prothrombin values, specifically deter mined (14) in patients on oral anticoagulant treatment, with values of other methods for sepa rate or several coagulation factors influenced by the treatment.

In our investigation all methods (specific assays of prothrombin, factors IX and X, Thrombotest and P-P) were well correlated but with values on

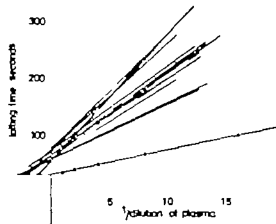


Fig 5 0 05 ml plasma or plasma dilution, added to 0 25 ml Thrombotest reagent. Clotting time obtained plotted against /dilution of plasma. □, Plasma from patients ith prothrombin/Thrombotest ratio 3 1 to 5 1 Mean alues from 10 patients on long term anticoagulant treatment Thin lines represent values from separate patients. ● Plasma from patient with prothrombin Thrombotest ratio 1.5 1 to 1 ○ Normal plasma (mixed from 20 healthy subjects).

Table III. *Patients with minor bleeding symptoms during coumarin treatment*

Inderal® (propranololchlor.), ICI, Macclesfield, England. Sulfapral (sulfamethizole + sulfamethoxypyridazine), Astra, Södertälje, Sweden. Furadantin® (nitrofurantoin), Pharmacia, Uppsala, Sweden. Doleron (acetylsalicylic acid + dextropropoxyphen + phenazon + diethylaminoethylphenothiazincarboxylchlor + butenemal), Astra. Ekvacillin (cloxacillinsodium), Astra Lasix® (N-furylmethylchlorsulfamoylanthranilic acid), Hoechst, Frankfurt, West-Germany Aldactone (spironolactone), Searle, Chicago, USA. Lanacrist (digoxin), Draco, Lund, Sweden. Kaliftabs (potassium chlor.), Ferrosan, Malmö, Sweden. Nitropent (pentanitrol), Astra. Sedisonal® (allypropymal + codein + acetylsalicylic acid + phenacetin), Leo, Helsingborg, Sweden. Nicangin® (nicotinic acid), Draco. Vasculat (bamethansulph.), Boehringer/Ingelheim, West-Germany Paranyl (theophylline + theobromine + caffeine + pentymal + ephedrine chlor.), Hässle, Gothenburg, Sweden. Duphaston (pheny thole), Leo. Pronestyl® (procaine chlor.), Squibb, New York, USA.

Pat. no.	Complicating disease	Bleeding symptoms	Medication	Prothrombin (of normal)	Thrombotest (of normal)
1	—	Hematemesis	—	11	<5
2	—	Hematuria	—	14	<5
3	Colitis	Melena	Inderal	14	7
4	Cancer vesic. urinar.	Hematuria[a]	Sulfapral Furadantin	15 28	6 8
5	—	Hematoma	Doleron[d] Ekvacillin	19	7
6	Diabetes	Nose bleeding	Lasix Aldactone Lanacrist Kaliftabs Nitropent	26	5
7	—	Rectal bleeding	Doleron Sedisonal Nicangin Vasculat	29	8
8	Asthma bronch.	Nose bleeding	Paranyl	29	11
9	—	Metrorrhagia[b]	Duphaston Pronestyl	40	8
10	Bronchitis chron.	Nose bleeding[c]	None	43	11

[a] Bleeding started after radiation treatment. Platelets 170 000–195 000/µl
[b] Platelets 166 000/µl
[c] Frequent nose bleedings since childhood
[d] In high dosage

different activity levels. Prothrombin values of 20–45% corresponded to Thrombotest values of 5–70% and P P values of 10–25%.

According to Loeliger et al. (12) factors II, VII, IX and X are similarly depressed during long-term anticoagulant treatment. Owren (19) found factor X activity considerably below that of factors VII, IX and prothrombin. Our results are in accordance with the findings of Owren, although we have registered somewhat higher factor X activity, probably due to the use of another method.

It has been shown that the marked depression of Thrombotest and factor X values, as compared to prothrombin and P P values, is caused by an inhibitor of prothrombin activation occurring during coumarin treatment (7), presumably a changed non-reactive prothrombin molecule (5). The P P method and Thrombotest show different relations to prothrombin values (Figs. 3 and 4) mainly due to the different sensitivity to the inhibitor (9, 11). The importance of this inhibitor for the bleeding risk of the patient was further investigated by studying patients with complications during anticoagulant treatment.

The frequency of bleeding and thrombotic complications was investigated in a large number of patients on long-term anticoagulant treatment. About 1.5% of the patients had minor bleeding symptoms. None of the patients reported thrombotic symptoms. The patients with complications were investigated with the specific prothrombin method besides Thrombotest, which was otherwise used for the control of the treatment. Several patients with bleedings had values in the therapeutic range, and about the same number with both methods (Table III). Most of the patients with bleedings had additional medication or suffered from diseases which could cause an increased bleeding tendency.

The activity of the inhibitor was determined according to Hemker et al. (7) and was found to be not higher but slightly lower in patients with bleeding complications than in other patients.

In patients with low inhibitor activity the Thrombotest values will be less depressed and consequently relatively higher than in other patients. Two such patients were found among the patients with treatment difficulties (group II) but they did not show an increased bleeding tendency if kept on Thrombotest values above 7%. Hence the differences in Thrombotest values caused by varying inhibitor activity are probably not of clinical importance. However, as the inhibitor influences some test methods but probably not coagulation in vivo, it would seem proper to use a method with low sensitivity to the inhibitor.

The value of a specific prothrombin method for the control of anticoagulant treatment, aiming at a slight depression of prothrombin, has been pointed out (17). From our investigation it seems possible to use the present specific prothrombin assay for the control of any type of oral anticoagulant treatment.

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PLASMA LIPIDS AND LIPOPROTEINS IN GREENLANDIC WEST COAST ESKIMOS

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Abstract. The plasma lipid and lipoprotein pattern has been examined in 130 Greenlandic West coast Eskimos with a life pattern, and especially with dietary habits, rather close to those of original Eskimos and essentially different from those of West Europeans. The age groups of the persons examined were chosen so as to be comparable with the age groups in which ischaemic heart disease prevails in West Europeans. The results of determination of plasma lipid and lipoproteins in the 130 Greenlandic west coast Eskimos, 69 women and 61 men, were compared with those of a similar examination in Denmark, including 25 female Eskimos living in Denmark. Significantly lower concentrations of total lipids, cholesterol, triglycerides, β-lipoproteins and pre-β-lipoproteins were found in Eskimos compared with Danish controls. The study on the Eskimos living in Denmark revealed plasma lipid values significantly higher than in Eskimos in Greenland and equal to those of Danish controls. This points strongly against a genetic and towards an environmental cause of the low lipid and lipoprotein concentrations in Greenlandic Eskimos. As in other societies with low plasma lipid concentrations, coronary atherosclerosis is rare among Greenlandic Eskimos. The Eskimos have low plasma lipid concentrations in spite of a high dietary intake of fat. This could be explained by the special composition of the Eskimo diet, with low content of saturated and high content of unsaturated fatty acids. Besides *coronary atherosclerosis*, diabetes mellitus is very rare among Eskimos. A correlation of this to the very low concentration of plasma triglycerides and pre-β lipoproteins is tempting, but purely speculative.

During the last decades studies of lipid metabolism have been closely connected with research in the field of atherosclerosis. It can now be considered a fact that a high serum lipid level, especially of cholesterol, is connected with high risk of ischaemic heart disease (16). Furthermore several studies of the serum lipid level in populations with low incidence of occlusive coronary disease have resulted in the finding of low serum lipid levels (21). Populations with predominantly vegetable dietary habits are likely to have low serum lipid levels as compared with the populations of the western countries (15) where, during the last decades, the animal protein and fat intake has increased in parallel with the incidence of ischaemic heart disease.

Ischaemic heart disease is, on the contrary rare in Greenlandic Eskimos in spite of the extraordinarily high intake of protein and fat of animal origin in these people. Little is known about the plasma lipid levels in Greenlandic Eskimos. Sagild (20) found rather low cholesterol concentrations in serum from Eskimos from different parts of Greenland. The serum cholesterol level of Eskimos in the UmanaK district was found to be significantly lower than that of the Eskimos living in the much more urbanized Godthaab area. However more detailed lipid studies have not been carried out in Greenland up to the present time.

The very low incidence of ischaemic heart disease and diabetes mellitus in the UmanaK district of Greenland is very remarkable. During the 5-year period 1963–67 only three cases of atherosclerotic heart disease were registered, and not a single case of diabetes mellitus (3). (After conclusion of the manuscript we got the opportunity of seeing the records of the three cases mentioned. One of them suffered from rheumatic heart disease. The two others were two admittances of a 78-year-old man suffering from degenerative heart disease with BBB.) It must be remembered that the mean age of Eskimos is lower than that of West Europeans, but the difference is diminishing. The absence of diabetes mellitus is especially remarkable when considering the mixing of the Eskimo population with other races as described below

The genetic character of the Greenlandic Eskimos

During some 40 centuries Eskimos have repeatedly invaded Greenland, at first exclusively from North America, but more recently also from East Asia. The Eskimos spread southwards on the west and east coasts of Greenland. During the 10th and the following centuries Scandinavians —predominantly Norwegians and Icelanders—invaded Greenland and settled on the southern part of the west coast, but later on spread northwards. Remnants of their buildings and tools are found as far to the north as the UmanaK district. These Scandinavians disappeared in the 15th century but in the 18th century a new invasion of Scandinavians started and is still going on. Consequently the possibility of a mixing of the races has existed for almost 1 000 years, predominantly in the southern parts of Greenland and fading northwards. It is probable that even in the district of UmanaK the existence of pure Eskimos is rare. During recent years examinations of the blood groups of Eskimos have confirmed this opinion.

However the "watering" of the Eskimo element has not gone so far as to extinguish the very typical Eskimo appearance. About half the population of the UmanaK district is still of small height, black-haired with rather dark complexion narrow eyes, high cheekbones and broad noses. Fair-haired persons are exceptions.

In the present paper the plasma lipid and lipo- concentrations in 130 Greenlandic west st Eskimos will be compared with the levels of the same components in a Danish population consisting of 316 persons, including 25 female Greenlandic Eskimos living in Denmark.

MATERIAL AND METHODS

For a study of serum lipids in Greenland population still living mainly in the original way especially concerning dietary habits, should be chosen. The number of the population should be sufficiently large to provide representative material, and there should be facilities allowing the establishment of lipid laboratory in the area. The travelling distances between this laboratory and the settlements should not be too long. The UmanaK district was considered to be the area best fulfilling these conditions.

The expedition numbered two medical doctors and one laboratory technician. As lipoprotein electrophoresis has to be carried out with fresh plasma (i.e. within 10 hours after venipuncture), complete equipment for handling the blood specimens and performing lipoprotein electrophoresis was sent to UmanaK in advance. The equipment was installed in the small laboratory of the local hospital, and arrangements were made to provide running cooling water for the electrophoresis, using ice from the fiord.

August and September were considered the best months for the visits to the settlements, as the weather and ice conditions in the fiords are generally best for sailing during this part of the year. Furthermore the hunting and fishing are usually best during the late summer and consequently the dietary habits of the population should be typical at this time.

The populations of the small settlements were preferred for the investigation, as their living patterns were more primitive than those of the population of the town of UmanaK. The population of the settlements ranged from about 80 to 300 persons. As the aim of the investigation as a study of the plasma lipid pattern of healthy adult people as compared with that of Europeans of the age groups in which ischaemic heart disease is prevalent, the material consisted of 61 males and 69 females, aged more than 30 years, and living in six settlements (Ikerasak, Qarsut, Niaqornat, Igdlorssuit, Satut, and Uvkusigsat). The age and sex distribution of the examined persons is given in Table I.

People ere asked about their dietary habits and any major diseases. The individuals were measured and weighed, and their BP was registered. Venipuncture as carried out in the morning after 10–12 hours of complete fasting. Serum or plasma was immediately separated by (hand) centrifugation and kept cooled in ice-water during transportation to the hospital of UmanaK, here the lipid laboratory had been established. No transportation exceeded 10 hours, and the lipoprotein lectrophoresis was realised immediately after return from the settlements.

The rest of the serum and plasma specimens ere kept refrigerated at −20°C, until the chemical lipid assays could be carried out after the return to Denmark.

The concentrations of the following lipids were determined total plasma lipid gravimetrically after Folch extraction (11), total plasma cholesterol by Liebermann-Burchard method (19), plasma triglycerides after Eggstein and Kreutz (10), plasma phospholipid as lipid phosphorus (2). The fatty acids of the cholesterol esters, triglycerides and phospholipids ere determined quantitatively by gas liquid chromatography after interesterification and methylation (12). The results of this part of the examination will be published separately (4).

Lipoprotein electrophoresis was carried out on agarose gel and estimated quantitatively after Dyerberg and Hjørne (6, 7, 8).

The UmanaK district and its population

The UmanaK district is situated at the 71st latitude about 500 km north of the Arctic Circle on the west coast of Greenland. It consists of the town of UmanaK with about 1 000 inhabitants and of seven small settlements, mostly on islands within distance of about 100 km from UmanaK and with further 1 300–1 400 inhabitants in all. While several of the inhabitants of the town of UmanaK are employed in public work (trade, engineering,

Table I. *Age and sex distribution of the examined Eskimos in Greenland*

Age group		Mean age (y.)
Men		
31–40	16	36.4
41–50	11	45.1
51–60	14	56.1
> 61	20	68.4
Total	61	53.0
Women		
31–40	19	36.5
41–50	16	45._
51–60	15	54.5
> 61	19	66.8
Total	69	50.8

shipping, etc.), the population of the small and very scattered settlements consists predominantly of hunters and or fishermen and their families.

Seals and sea birds are the prime objects of hunting. Now and then small whales (Lesser Rorqual, Beluga, Narwhale) are landed. The fishing is mostly Greenlandic halibut and some types of trout. Meat and fish constitute the main nutrition of the population being often eaten without addition of potatoes or other vegetables, mostly boiled, sometimes raw. The intake of protein and fat is consequently extremely high as compared with that of Europeans, the carbohydrate intake being correspondingly low. August Krogh, who in 1908 carried out metabolic studies on Eskimos of the Island of Disko close to the UmanaK district, stated: "The Eskimos are probably the most exquisitely carnivorous people on earth, living, as most of them do, almost exclusively on meat and fish." And further: "The normal diet of Eskimos contains an excessive amount of animal protein (280 gr.) and much fat (135 gr.) while the quantity of carbohydrate is extremely small (54 gr. of which more than is derived as glycogen from the meat eaten). Their dietary habits are very like those of the carnivorous animals" (17). A survey of the diet of Eskimos is given by Sinclair (22). The intake of dairy products is very low.

Hunting statistics of Greenland are published every year on the number of seals and whales caught and the average weight of meat and edible entrails of the animals. The figures from 1970 (the year of the expedition) from the UmanaK district are given in Table II. As only a small number of the animals are sold outside the district (in the year 1970 1 910 kg of whale and no seal meat), an estimate is possible concerning the amount of seal and whale available per individual and day though very rough because of the variability in weight of the seals and whales. The amount of seal and whale meat consumed in 1970 may be estimated at nearly 0.4 kg per individual and day. To this must be added the unknown and sometimes not small amount of bird and fish meat. The fish hunt, predominantly Greenlandic halibut and trout, is not registered, but during certain parts of the year this sort of food is rather important. Of less importance is the bird hunting.

Conclusively it may be stated that the food of the population in the small settlements in the UmanaK district is still mainly of animal origin and consequently very rich in protein and fat. The intake of carbohydrates is very much lower than that of the West Europeans. During our interviews with the individuals in our study this impression of the composition of their food was very clearly confirmed.

Table II. *Captured sea animals in the UmanaK district in 1970 and calculations of the meat consumed from these sources*

	Whales			Seals				
	Lesser Rorqual[a]	Narwhale[b]	Beluga[c]	Ringed seal[d]	Bearded seal[e]	Harp seal[f]	Hooded seal[g]	Total
Animals captured (n)	22	23	8	11 904	78	476	75	12 536
Average weight of meat and edible entrails (kg)	2 000	250	225	22	110	35	100	
Total weight of meat and edible entrails (kg)	44 000	5 750	1 800	261 888	7 080	16 660	7 500	340 678
Meat sold outside the UmanaK district (kg)	1 900							1 900
Meat and entrails consumed in the UmanaK district (kg)	42 100	5 750	1 800	261 888	3 080	16 660	7 500	338 778
Estimated available whale and seal meat per person (2 400 inhabitants) and day (kg)								0.387

[a] Balaenoptera acutorostrata L., [b] Monodon monoceros L., [c] Delphinapterus leucas P., [d] Pusa hispida S., [e] Erignathus barbatus E., [f] Pagophilus groenlandicus E., [g] Cystophora cristata E.

Table III *Concentration of plasma total lipids and cholesterol in Greenlandic Eskimos as compared with Danish controls*

Figures | Italics are logarithmic values. $\bar{x}$ = mean value, S.D = standard deviation, $S.D._{\bar{x}}$ = standard deviation of the mean

Age (y)	Total lipids (g/l) Eskimos $\bar{x}$	S.D	$S.D._{\bar{x}}$	Danes $\bar{x}$	S.D	$S.D._{\bar{x}}$	Sign. of diff	Cholesterol (mmol/l) Eskimos $\bar{x}$	S.D	$S.D._{\bar{x}}$	Danes $\bar{x}$	S.D	$S.D._{\bar{x}}$	Sign. of diff.
Males														
31–40	6.01	0.99	0.25	6.29	0.74	0.15	–	5.58	0.75	0.19	6.28	0.94	0.19	$p<0.02$
	0.773	*0.073*	*0.018*	*0.769*	*0.052*	*0.010*		*0.743*	*0.059*	*0.015*	*0.793*	*0.065*	*0.013*	
41–50	6.42	1.17	0.35	7.42	1.63	0.31	$p<0.05$	6.18	1.05	0.32	7.31	1.47	0.28	$p<0.02$
	0.801	*0.077*	*0.023*	*0.861*	*0.093*	*0.018*		*0.785*	*0.074*	*0.022*	*0.855*	*0.088*	*0.017*	
51–60	6.46	1.02	0.27	7.17	1.16	0.19	p 0.05	6.56	1.05	0.28	7.26	1.15	0.19	$p<0.05$
	0.805	*0.072*	*0.019*	*0.850*	*0.069*	*0.012*		*0.812*	*0.067*	*0.018*	*0.855*	*0.070*	*0.012*	
≥61	5.95	0.56	0.13	7.04	1.28	0.28	$p<0.005$	5.93	0.88	0.20	7.00	1.44	0.31	$p<0.01$
	0.772	*0.042*	*0.009*	*0.841*	*0.079*	*0.017*		*0.768*	*0.068*	*0.015*	*0.836*	*0.091*	*0.020*	
Females														
31–40	5.71	0.93	0.21	6.32	0.97	0.22	$p<0.05$	5.26	1.25	0.29	6.44	1.18	0.26	$p<0.005$
	0.731	*0.072*	*0.017*	*0.796*	*0.064*	*0.014*		*0.710*	*0.100*	*0.023*	*0.801*	*0.084*	*0.019*	
41–50	6.18	0.91	0.23	6.77	1.05	0.23	–	5.96	1.52	0.38	7.09	1.32	0.29	$p<0.02$
	0.787	*0.065*	*0.016*	*0.826*	*0.066*	*0.014*		*0.763*	*0.107*	*0.027*	*0.844*	*0.080*	*0.018*	
51–60	6.50	0.89	0.23	8.02	1.29	0.29	$p<0.001$	5.83	0.84	0.22	8.32	1.24	0.28	p 0.001
	0.809	*0.061*	*0.016*	*0.899*	*0.070*	*0.016*		*0.761*	*0.063*	*0.016*	*0.915*	*0.067*	*0.015*	
≥61	6.21	0.86	0.20	8.19	1.26	0.27	p 0.001	5.98	0.87	0.20	8.45	1.58	0.34	$p<0.001$
	0.789	*0.058*	*0.013*	*0.909*	*0.067*	*0.014*		*0.772*	*0.061*	*0.014*	*0.920*	*0.079*	*0.017*	

STATISTICAL METHODS

The results of the investigation were grouped according to age and sex and examined statistically for the frequency distribution of the parameters. A similar examination of Danish normal material has been described previously (9). The values were tested for fitness to the normal distribution by the Kolmogorov-Smirnov one-sample test, before and after logarithmic transformation of the values. The fitness of the values to the normal distribution was uniformly rather good. However, the fitness to the log-normal distribution, too, was excellent, which allowed comparison with the Danish normal material, using Student's t-test on the values after logarithmic transformation. In situations where parametric statistical tests could not be used, non-parametric statistical tests as described previously (9) were used.

The results obtained in the Greenlandic Eskimos were compared with those of 25 female Eskimos living in Denmark, aged 27–51 years, mean age 38.0 years, and with healthy Danes in the corresponding age groups. The analytical techniques used in the two control materials were identical with those of the Greenlandic Eskimos.

RESULTS

The lipid and lipoprotein values of the Greenlandic Eskimos compared with those of a Danish control material in the same age groups (9) are given in Tables III–VI.

Variation of plasma lipids and lipoproteins related to sex and age

Only minor sex differences in the lipid and lipoprotein concentrations were found. Generally the cholesterol and triglyceride concentrations were a little higher in men than in women but this difference was only significant for cholesterol in the age group 51–60 years ($p<0.05$) and for triglycerides in the age group 31–40 years ($p<0.01$). When all the age groups were combined, the following values were obtained for men and women, respectively: triglycerides 0.64 ± 0.32 and 0.50 ± 0.15 mmol/l; cholesterol 6.03 ± 0.91 and 5.74 ± 1.11 mmol/l (mean ± S.D.). The difference for triglyceride concentrations was significant ($p<0.005$) while it was insignificant for the cholesterol concentrations. No significant sex difference could be demonstrated for any of the other lipid and lipoprotein parameters.

The rather limited total age range did not allow extensive study of variations in lipid and lipoprotein concentrations related to age. No significant variation in men could be demonstrated for any of the parameters, except cholesterol, for which a significant increase with age was found up to 51–60 years, after which age

Table IV *Concentration of plasma triglycerides and phospholipids in Greenlandic Eskimos as compared with Danish controls*

Figures in italics are logarithmic values. Abbreviations as in Table III

Age (y.)	Triglycerides (mmol/l) Eskimos $\bar{x}$	S.D.	S.D.$_{\bar{x}}$	Danes $\bar{x}$	S.D.	S.D.$_{\bar{x}}$	Sign. of diff	Phospholipids (mmol/l) Eskimos $\bar{x}$	S.D.	S.D.$_{\bar{x}}$	Danes $\bar{x}$	S.D.	S.D.$_{\bar{x}}$	Sign. of diff
Males														
31–40	0.66	0.44	0.11	1.15	0.53	0.11	p<0.001	2.94	0.50	0.12	2.75	0.44	0.09	–
	−0.239	*0.209*	*0.052*	*0.023*	*0.180*	*0.036*		*0.462*	*0.072*	*0.018*	*0.434*	*0.070*	*0.014*	
41–50	0.62	0.26	0.08	1.65	0.79	0.15	p<0.001	2.99	0.51	0.15	2.91	0.85	0.16	–
	−0.239	*0.175*	*0.053*	*0.177*	*0.183*	*0.035*		*0.469*	*0.077*	*0.023*	*0.444*	*0.138*	*0.027*	
51–60	0.61	0.21	0.06	1.3[illegible]	0.62	0.10	p<0.001	3.42	0.55	0.15	2.84	0.60	0.10	p<0.005
	−0.238	*0.148*	*0.039*	*0.079*	*0.183*	*0.031*		*0.579*	*0.069*	*0.018*	*0.444*	*0.100*	*0.017*	
>61	0.68	0.32	0.07	1.35	0.81	0.18	p<0.001	3.14	0.49	0.11	2.79	0.57	0.13	p<0.05
	−0.206	*0.189*	*0.042*	*0.065*	*0.240*	*0.052*		*0.492*	*0.067*	*0.015*	*0.437*	*0.094*	*0.021*	
Females														
31–40	0.41	0.16	0.04	0.95	0.40	0.09	p<0.001	2.82	0.64	0.15	2.83	0.6[illegible]	0.14	
	−0.426	*0.184*	*0.042*	*−0.061*	*0.197*	*0.044*		*0.441*	*0.091*	*0.021*	*0.440*	*0.106*	*0.024*	
41–50	0.47	0.14	0.03	1.02	0.43	0.09	p<0.001	3.05	0.85	0.21	2.98	0.40	0.09	
	−0.351	*0.149*	*0.037*	*−0.028*	*0.181*	*0.039*		*0.467*	*0.128*	*0.032*	*0.471*	*0.056*	*0.012*	
51–60	0.54	0.19	0.05	1.10	0.41	0.09	p<0.001	3.27	0.46	0.12	3.28	0.47	0.11	–
	−0.293	*0.156*	*0.040*	*0.007*	*0.185*	*0.041*		*0.510*	*0.062*	*0.016*	*0.511*	*0.064*	*0.014*	
>61	0.59	0.15	0.03	1.30	0.47	0.10	p<0.001	2.94	0.33	0.08	3.30	0.54	0.12	p<0.025
	−0.243	*0.109*	*0.025*	*0.036*	*0.166*	*0.035*		*0.466*	*0.049*	*0.011*	*0.512*	*0.075*	*0.016*	

Table V *Concentration of plasma chylomicrons and pre-β-lipoproteins in Greenlandic Eskimos as compared with Danish controls*

Figures in italics are logarithmic values. Abbreviations as in Table III

Age (y.)	Chylomicrons (g/l) Eskimos $\bar{x}$	S.D.	S.D.$_{\bar{x}}$	Danes $\bar{x}$	S.D.	S.D.$_{\bar{x}}$	Sign. of diff	Pre-β-lipoproteins (g/l) Eskimos $\bar{x}$	S.D.	S.D.$_{\bar{x}}$	Danes $\bar{x}$	S.D.	S.D.$_{\bar{x}}$	Sign. of diff
Males														
31–40	0.31	0.18	0.05	0.18	0.10	0.02	p<0.05	0.62	0.36	0.09	1.17	0.59	0.12	p<0.005
	−0.605	*0.326*	*0.081*	*−0.840*	*0.319*	*0.064*		*−0.260*	*0.244*	*0.061*	*0.011*	*0.[illegible]38*	*0.048*	
41–50	0.30	0.27	0.08	0.23	0.11	0.02	–	0.44	0.42	0.13	1.81	0.87	0.17	p<0.001
	−0.703	*0.452*	*0.136*	*−0.704*	*0.271*	*0.052*		*−0.627*	*0.405*	*0.182*	*0.213*	*0.201*	*0.039*	
51–60	0.30	0.12	0.03	0.16	0.08	0.01	p<0.001	0.42	0.28	0.07	1.58	0.77	0.13	p<0.001
	−0.540	*0.213*	*0.057*	*0.830*	*0.243*	*0.041*		*0.546*	*0.485*	*0.130*	*0.134*	*0.276*	*0.046*	
>61	0.28	0.12	0.03	0.21	0.13	0.03	–	0.30	0.27	0.06	1.45	1.02	0.22	p<0.001
	−0.592	*0.226*	*0.051*	*−0.715*	*0.227*	*0.050*		*0.741*	*0.519*	*0.116*	*0.072*	*0.285*	*0.062*	
Females														
31–40	0.36	0.18	0.04	0.16	0.10	0.02	p<0.005	0.45	0.30	0.07	0.99	0.54	0.12	p<0.005
	−0.527	*0.306*	*0.070*	*−0.889*	*0.379*	*0.074*		*−0.455*	*0.364*	*0.083*	*−0.093*	*0.325*	*0.073*	
41–50	0.18	0.10	0.03	0.15	0.09	0.02	–	0.43	0.35	0.09	1.11	0.49	0.11	p<0.001
	−0.810	*0.281*	*0.070*	*−0.833*	*0.198*	*0.041*		*−0.566*	*0.497*	*0.124*	*0.004*	*0.197*	*0.043*	
51–60	0.23	0.14	0.04	0.19	0.10	0.02	–	0.39	0.39	0.10	0.98	0.55	0.12	p<0.005
	−0.738	*0.333*	*0.092*	*−0.785*	*0.304*	*0.068*		*−0.647*	*0.353*	*0.143*	*−0.091*	*0.299*	*0.067*	
>61	0.21	0.10	0.02	0.23	0.08	0.02	–	0.42	0.31	0.07	1.23	0.68	0.14	p<0.001
	−0.726	*0.202*	*0.046*	*−0.633*	*0.144*	*0.031*		*−0.494*	*0.382*	*0.088*	*0.028*	*0.280*	*0.055*	

Table VI. *Concentration of plasma β- and α-lipoproteins in Greenlandic Eskimos as compared with Danish controls*

Figures in italics are logarithmic values. Abbreviations as in Table III

Age (yr)	β-lipoproteins (g/l) Eskimos x̄	S.D.	S.D.x̄	Danes x̄	S.D.	S.D.x̄	Sign. of diff.	α-lipoproteins (g/l) Eskimos x̄	S.D.	S.D.x̄	Danes x̄	S.D.	S.D.x̄	Sign. of diff.
Males														
31–40	4.32	0.88	0.22	4.55	0.85	0.17	–	3.47	1.02	0.26	2.90	0.69	0.14	–
	0.627	*0.091*	*0.023*	*0.651*	*0.080*	*0.016*		*0.505*	*0.221*	*0.045*	*0.449*	*0.107*	*0.021*	
41–50	4.58	1.56	0.47	5.19	1.52	0.29	–	4.20	1.54	0.46	2.84	1.08	0.21	$p<0.01$
	0.638	*0.146*	*0.044*	*0.699*	*0.1 1*	*0.023*		*0.596*	*0.166*	*0.050*	*0.4 4*	*0.165*	*0.032*	
51–60	4.52	0.70	0.19	5.32	1.08	0.18	p 0.005	4.41	1.61	0.43	2.78	0.89	0.15	$p<0.001$
	0.631	*0.063*	*0.017*	*0.718*	*0.083*	*0.014*		*0.615*	*0.167*	*0.045*	*0.423*	*0.136*	*0.033*	
≥61	4.23	0.71	0.16	5.22	1.20	0.26	$p<0.005$	4.09	1.51	0.34	2.75	0.80	0.18	p 0.01
	0.620	*0.076*	*0.017*	*0.707*	*0.098*	*0.021*		*0.573*	*0.209*	*0.047*	*0.424*	*0.117*	*0.026*	
Females														
31–40	4.03	1.00	0.23	4.29	1.23	0.23	–	3.31	1.20	0.28	3.71	0.92	0.21	–
	0.590	*0.121*	*0.028*	*0.616*	*0.1 4*	*0.028*		*0.493*	*0.158*	*0.036*	*0.556*	*0.111*	*0.025*	
41–50	4.56	0.74	0.19	4.86	1.22	0.27	–	4.03	1.51	0.38	3.57	1.10	0.24	–
	0.654	*0.072*	*0.018*	*0.673*	*0.110*	*0.024*		*0.579*	*0.153*	*0.038*	*0.534*	*0.131*	*0.029*	
51–60	4.48	0.81	0.21	6.02	1.45	0.32	$p<0.001$	4.74	1.90	0.49	4.35	1.04	0.23	–
	0.645	*0.079*	*0.020*	*0.767*	*0.107*	*0.024*		*0.632*	*0.223*	*0.058*	*0.627*	*0.104*	*0.023*	
≥61	4.88	1.16	0.27	6.26	1.52	0.32	$p<0.005$	3.74	1.19	0.27	3.78	0.97	0.21	–
	0.677	*0.105*	*0.024*	*0.785*	*0.100*	*0.0 1*		*0.551*	*0.142*	*0.035*	*0.563*	*0.119*	*0.0 5*	

the values declined again. In women a significant increase with age was found for cholesterol, triglycerides and β-lipoproteins. Phospholipids and α-lipoproteins increased significantly with age up to 51–60 years, after which age the values declined.

Comparison of plasma lipid and lipoprotein concentrations in Danes and Eskimos

Statistical comparisons with probability levels are given in Tables III–VI. Significantly lower values in the Eskimos were found for total lipids, cholesterol, triglycerides, pre-β-lipoproteins, and β-lipoproteins when compared with age- and sex-matched Danish control groups. The only exception was for β-lipoproteins in the two youngest age groups of both sexes, and for total lipids in the youngest male age group and in the female age group 41–50 years. Phospholipids showed significantly higher levels in Eskimos than in Danes in the two oldest male age groups and in the oldest female age group. α-lipoproteins were generally higher in male Eskimos than in the Danish controls, and this difference was significant except in the youngest age group. In Eskimo women the α-lipoprotein levels did not differ significantly from the Danish controls. Chylomicrons as a whole were a little higher in Eskimos than in Danes, but the absolute levels as well as the differences were small, with no systematically significant differences.

Comparison of lipid and lipoprotein concentrations in Eskimos living in Denmark and in Greenland

As it was possible to examine only female Eskimos living in Denmark, a group of 25 of them was compared with groups of female Eskimos living in Greenland and Danish women of the same age. The values from this examination are given in Table VII. The Eskimo women living in Denmark were aged 27–51 years (mean 38.0, S.D. 5.17). The median values for plasma total lipids, cholesterol, triglycerides, phospholipids, β-lipoproteins, and pre-β-lipoproteins were all significantly higher in Eskimos living in Denmark than in Eskimos living in Greenland. Chylomicrons did not show any difference, and the difference in α-lipoproteins was not significant ($p<0.10$, Wilcoxon rank sum test). In comparisons between Eskimos living in Denmark and Danish women, only minor differences were observed. As a whole the values for Eskimo women living in Denmark were a little higher than those of Danish women. Total lipids were

Table VII *Plasma lipid and lipoprotein concentrations in Eskimo women living in Greenland and in Denmark and in Danish women*

TL total lipids (g/l), C = cholesterol (mmol/l), TG = triglycerides (mmol/l), PL phospholipids (mmol/l), CH chylomicrons (g/l), B = β-lipoproteins (g/l), PB = pre-β-lipoproteins (g/l), A = -lipoproteins (g/l)
$\bar{x}$ mean value, S.D.$_{\bar{x}}$ = standard deviation of the mean

	Eskimos, Greenland (35 $\bar{x}$ = 40.6 y)			Eskimos, Denmark (n = 25 $\bar{x}$ = 38.0 y)			Danes (= 41 $\bar{x}$ 41.1 y)		
	$\bar{x}$	S.D.$_{\bar{x}}$	Median	$\bar{x}$	S.D.	Median	$\bar{x}$	S.D.	Median
TL	5.93	0.16	5.68	7.32	0.27	7.00	6.55	0.16	6.50
C	5.58	0.24	5.48	7.30	0.25	7.03	6.77	0.20	6.81
TG	0.43	0.03	0.42	1.12	0.10	1.10	0.98	0.06	0.97
PL	2.92	0.25	2.73	3.18	0.09	3.24	2.91	0.08	2.84
CH	0.27	0.03	0.24	0.24	0.02	0.21	0.15	0.02	0.16
B	4.27	0.16	4.29	5.00	0.25	4.92	4.58	0.19	4.34
PB	0.44	0.05	0.41	1.10	0.13	1.01	1.05	0.08	1.01
A	3.64	0.23	3.38	4.24	0.27	4.16	3.64	0.16	3.78

significantly higher reflecting significantly higher phospholipid concentration ($p < 0.025$) whereas the difference in the phospholipid-rich α-lipoprotein concentration was not significant ($p < 0.10$) No significant difference could be demonstrated in cholesterol, triglycerides, β- and pre-β-lipoprotein concentrations.

Relation of plasma lipid and lipoprotein concentrations to racial characteristics

Due to the unknown and varying degree of mixing of the Eskimos with other races, predominantly Scandinavians, an investigation was made of whether the lipid and lipoprotein concentrations had any relation to the typical Eskimo appearance Though it was well known that this stratification was rather coarse Ahlbirk (2) studying the same persons, found differences in the geometry of the eyes when they were divided into pure Eskimos and "mixed types" The division into these two groups was performed by two persons with years of experience of living among Eskimos in the UmanaK district. The values from this examination are given in Table VIII The only value showing any significant difference was β-lipoproteins with higher level in the mixed group than in the pure group" ($p < 0.025$).

Influence of overweight

Height and weight were recorded for all persons. A clear classification of overweight based on these parameters was, however not possible. The Danish controls were classified according to Natvig (18) and overweight was defined as a weight exceeding "the ideal weight + 15 % These figures are based on an examination of Scandinavians with a build of body differing from that of the Eskimos If however the Eskimos were classified according to Natvig's tables, a rather low frequency of overweight was found, in all 7 men and 7 women, which gives a common frequency of 10 % Four of the Eskimo women living in Denmark were obese when classified in the same manner Due to the doubtful nature of this classification the obese Eskimos were not excluded. However in the Danish control group, obese persons were excluded As plasma lipid values in obese persons are generally higher than in the non-obese (9) this classification will only tend to diminish the differences which have been demonstrated

DISCUSSION

The main results of the present investigation have been a demonstration of essentially lower levels of plasma lipids and lipoproteins in Eskimos living in Greenland than in people from a typical West European community" namely urbanized people living in Denmark. The difference was most pronounced for triglycerides and the triglyceride rich pre β-lipoproteins, but also remarkable for cholesterol and the cholesterol-rich β-lipoproteins. For the first two components the values in all age groups of Eskimos were even lower than

Table VIII *Comparison of the plasma lipid and lipoprotein values in "pure" and "mixed" Eskimos*

Abbreviations as in Table VII

	Pure (n = 59, $\bar{x}$ = 50.07 y, S.D = 11 74 y.)		Mixed (n = 71, $\bar{x}$ = 53.28 y, S.D = 13.52 y)	
	$\bar{x}$	S.D	$\bar{x}$	S.D.
TL (g/l)	6 05	0.91	6.23	0.93
C (mmol/l)	5.78	1 06	5.95	1 10
TG (mmol/l)	0 55	0.22	0 51	0.26
PL (mmol/l)	3.08	0 61	3.04	0.55
PB (g/l)	0.47	0 31	0 40	0.35
B (g/l)	4.21	1.00	4.59	0.84
A (g/l)	4.02	1.54	3.91	1 41

those found in the youngest Danish age group. In contrast thereto phospholipids and α-lipoproteins were generally a little higher in Eskimos than in Danes; this difference was, however significant only for men. Very few examinations of plasma lipids and no examinations of plasma lipoproteins in Greenlandic Eskimos have been carried out. Sagild (20) who examined serum cholesterol values in a great number of people living in Greenland found values comparable with ours, and with a marked tendency to higher levels in Greenlandic communities where the influence of the western way of life was most marked.

The reason for the differences in plasma lipid concentrations between Eskimos and West Europeans might be genetic or due to different living habits The way of life of the Eskimos examined in this study differs substantially from that of the Danish controls. The difference is mainly due to greater physical activity of Eskimos, and to dietary habits which are completely different in the two groups. The possible genetic influence is elucidated by the part of our study concerning Eskimos living in Denmark and the division of the Greenlandic Eskimos into groups of pure and "mixed" Eskimos. The plasma lipid and lipoprotein concentration in Eskimos living in Denmark did not differ from those of other inhabitants in Denmark, and the division of the Greenlandic Eskimos into pure and "mixed" types did not reveal any major difference between the groups. Taking the group of Eskimo women living in Denmark as a common exponent for Eskimos exposed to other environmental stimuli than those in Greenland, both examinations point strongly against a genetic and towards an environmental explanation of the difference in lipid and lipoprotein concentrations. Returning to the most pronounced differences in living habits in Greenland and Denmark, the differences in physical activity probably explain some of the differences in the lipid values. High physical activity seems to have some decreasing influence on plasma lipid and lipoproteins (5). It does not seem probable, however that the difference in physical activity which is not great especially in the older age groups, as the elderly Eskimos do not participate in the hunting and fishing could be the only explanation of the observed differences.

A survey of the composition of the Eskimo food in this part of Greenland has been given in this paper and it seems to us that, in spite of the high intake of fat of animal origin, the composition of the special Eskimo diet must be one of the main reasons for the low plasma lipid and lipoprotein concentrations in Eskimos. It is known that a high content of unsaturated fatty acids in the diet lowers the serum cholesterol level (14). The fatty acid composition of the fat consumed in North Greenland is partly unknown, but from studies of the fatty acid composition in fish and whale oil in other parts of the world it is most likely that the Eskimo diet must be low in saturated and high in unsaturated fatty acids These facts could be reflected in the fatty acid composition of the plasma lipids in the Eskimos; studies on this problem are in progress (4)

The generally accepted fact that high levels of plasma cholesterol and probably also of triglycerides (1) are correlated with high incidence of coronary atherosclerosis, and vice versa, is confirmed by our study as coronary atherosclerosis is a very uncommon disease among Greenlandic Eskimos (3) As the metabolism of triglycerides and pre-β-lipoproteins is closely related to the carbohydrate metabolism, it is tempting to correlate the very low concentrations of these two plasma lipid components with the fact that diabetes mellitus is nearly unknown among Eskimos. It should be pointed out that any causative relation is purely speculative, and no information of a metabolic link emerges from this study However in this connection the very low

carbohydrate intake by the Eskimos should be remembered.

It is interesting to compare the results of our study with those of Keys and Kimura (15) who examined serum cholesterol and triglyceride con centrations in Japanese farmers, aged 40–59 years. The diet of the Japanese farmers was extremely low in fat, about 8% and high in carbohydrate about 80%. This is in an absolute contrast to the diet of the Eskimos. As pointed out earlier no exact information exists about the composition of the Eskimo diet in this part of Greenland, but the amount of fat and carbohydrates may be estimated at about 50 and 20% respec tively of the daily caloric intake. The plasma concentrations of cholesterol and triglycerides in Keys' and Kimura's study were found to be 147 ± 30.5 and 98 ± 40.7 mg/100 ml, respectively (mean ± 1 S.D.). In comparison our values from Eskimo men, aged 41–60 years, were 47 ± 40 mg/100 ml for cholesterol and 55 ± 20 mg/100 ml for triglycerides. Both values differ significantly when using Student's *t*-test. Even if Student's *t*-test should be handled with great care in a situation like this, the very low probability level ($p < 0.001$) seems to justify this calculation. The conclusion of this comparison of two populations differing essentially in dietary habits, but with a very low incidence of coronary atherosclerosis, must be that the protective factors against coronary atherosclerosis—among many others—are a combination of low plasma cholesterol and triglycerides, which in this respect means low plasma β and pre-β-lipoprotein concentrations.

Another interesting observation was that plasma lipids and lipoproteins show much less correlation to age and sex in Greenlandic Eskimos than in Danes (9). Except for a minor but significantly higher triglyceride concentration in Eskimo men than in women, no other sex difference in the lipid parameters was found. The variation of the lipid parameters with age among Eskimos was small, but most pronounced in women, for whom a small but significant increase with age was found in cholesterol, triglyceride, β-lipoprotein, phospholipid, and α-lipoprotein concentrations. In men only cholesterol showed any significant age variation, with a maximum in the age group 51–60 years. It is possible, though no proof exists, that the small sex and age variations are due to the same mechanism as causes the low plasma lipid levels. The very similar levels of plasma lipids and lipoproteins in Eskimo women in Denmark and in Danish women of the same age point strongly towards this explanation.

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THE EFFECT OF FUROSEMIDE UPON RENAL OXYGEN CONSUMPTION IN THE HUMAN KIDNEY

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Abstract. The effect of an i. v. injection of furosemide upon the oxygen consumption and sodium reabsorption of the kidney has been investigated in ten patients. The injection of furosemide increased the excretion fraction of sodium from 1.96 to 14.43. Probably because of increased glomerular filtration rate, the absolute amount of sodium reabsorbed was not significantly reduced. No change of the oxygen consumption of the kidney and of the ratio between reabsorbed sodium and the oxygen consumption was observed. The extraction ratio of para-aminohippuric acid and the arterio-venous oxygen difference were unaffected by the injection.

The main part of the oxygen consumption of the kidney seems linked to the reabsorption of the glomerular filtrate and in particular to the reabsorption of sodium. In the normal dog kidney and in several experimental conditions in the dog a constant relation between the amount of sodium reabsorbed and the renal oxygen consumption has been observed (2, 5). A similar relation between reabsorbed sodium and oxygen consumption seems to be present also in the normal and diseased human kidney (8).

On the base of studies of cellular enzymes and kidney metabolism (6) it has been suggested that the energy required for the sodium reabsorption is provided by aerobic metabolic processes in the renal cortex and mainly by anaerobic metabolic pathways in the medulla. This might imply that the ratio between the amount of reabsorbed sodium and the oxygen used is not the same in the cortex as in the medulla. However, this has not been verified in recent experiments in the dog when the sodium transport in the loop of Henle was blocked by cyanide (6, 11) or ethacrynic acid (4) and the sodium reabsorption in the distal tubule was reduced by hydrochlorothiazide (9).

We have studied the effect upon the oxygen consumption of the human kidney of another potent diuretic, furosemide, which also mainly reduces the sodium reabsorption in the ascending limb of the loop of Henle (7) and the metabolic rate in the outer medulla of the dog (1). Our intention was 1) to measure the oxygen consumption of the kidney before and after an i. v. injection of furosemide with 2) special regard to the ratio between the amount of sodium reabsorbed and the oxygen consumption.

In clinical practice furosemide is often used in patients with reduced arterial oxygen saturation combined with a low renal blood flow compared with the amount of glomerular filtrate to be absorbed (high filtration fraction), the findings therefore were thought to be of potential practical value.

MATERIAL

Ten patients (six of them men), on average 54 years old, were investigated during their stay in hospital. In all patients diuretic was given for therapeutical reasons, in one patient because of heart failure, in the rest because of hypertension. In all patients i. v. pyelography was performed, signs or symptoms of asymetric renal disease or infection were not present. The renal function estimated by the clearance of inulin (C_{In}) and the extraction ratio of para-aminohippuric acid (E-PAH) was normal in all patients except one with hypertension and proteinuria, in whom the C_{In} was 51 ml/min and the E-PAH 0.69. The investigation was performed with the patients' consent.

METHODS

The investigation was carried out in hydrated patients. No diuretic therapy was given for the last week before the investigation. The patients were given a modified low sodium diet containing about 60 μEq of sodium per day

Table I. *Findings before (control values) and after (experimental values) i.v. injection of 10 mg furosemide in ten patients (mean values)*

	Control values	Experimental values
C_{In} (ml/min)	100.2	109.4
C_{PAH} (ml/min)	479	421
(*Diuresis* · 100)/C_{In}	2.7	15.6
E PAH	0.85	0.85
Renal blood flow (ml/min)	1 015	1 312
Excretion fraction of Na ( )	1.96	14.43
Na reabsorbed (μEq/min)	13.4	12.4
Oxygen consumption (ml/min)	22.4	4.8
Na reabsorbed (μEq/min) / Oxygen consumption (μmol/min)	13.7	12.1
Filtration fraction (C_{In}/C_{PAH})	0.212	0.180

*Statistically significant difference ($p < 0.02$).

The method of catheterizing the renal vein, the procedure of hydration, clearance measurements and the method of measuring the oxygen consumption have been described elsewhere (8).

After two control periods 10 mg of furosemide (Lasix®) was injected i.v. The following period of about 15 min was not used in the calculations because of the wash-out effect. The next two periods were used as experimental periods. The values given above are not corrected for BSA. The t-test of paired samples was used for the statistical analysis of the data.

RESULTS

The injection of furosemide reduced the percental reabsorption of sodium from 98.04 to 85.57 (Table I) with a corresponding increase of the diuresis and the osmolal clearance (not shown).

The amount of sodium reabsorbed was, however, not significantly reduced, probably because of a slight increase of the glomerular filtration rate. There was neither a significant change of the oxygen consumption nor of the ratio between the amount of sodium reabsorbed and the oxygen consumption. Neither was the E-PAH nor the arterio-venous oxygen difference significantly affected by the injection.

The correlation between the amount of sodium reabsorbed and the oxygen consumption is given in Fig. 1.

DISCUSSION

The oxygen consumption of the kidney probably serves two different functions: 1) the basal metabolic processes not linked to the reabsorption of the filtrate and 2) the metabolic processes providing energy for the sodium reabsorption.

The present study shows that the dose of furosemide usually given i.v. to patients is without measurable effect on the oxygen consumption of the kidney.

The dose used was too small to reduce the total amount of reabsorbed sodium significantly although the sodium excretion in the patients increased several times. The more drastic experimental conditions needed for this purpose—constant infusion of a high dose of furosemide (and bleeding of the experimental subjects to anemia to increase the arterio-venous oxygen saturation difference)—are probably not suited for clinical research. As a consequence the conclusions to be drawn are somewhat limited by the fact that the methodological errors in measuring the parameters involved were probably relatively great compared to the objects of measurement. With these reservations the experimental situation in

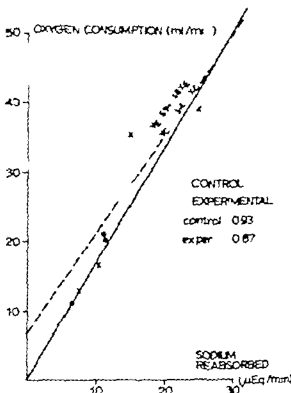


Fig. 1. Relation between oxygen consumption and reabsorbed sodium before (control values) and after (experimental values) i.v. injection of 10 mg furosemide. r = correlation coefficients, E = experimental values, C = control values.

this study was rather unique presenting a reduced reabsorption fraction of sodium combined with a constant amount of sodium reabsorbed. The finding of an unchanged oxygen consumption and a constant relation between reabsorbed sodium and the oxygen consumed, therefore, indicates that furosemide does not greatly influence the relation between reabsorbed sodium and the oxygen consumption. This corresponds with the findings of Vorburger (10) in five patients.

If it is assumed that the basal oxygen consumption is unaffected by furosemide and that the injection of furosemide does not change the stoichiometric relation between sodium reabsorption and oxygen consumption, one might expect a reduction of the ratio between reabsorbed sodium and the oxygen consumption because the relative part of the total oxygen consumption used for basal metabolic purposes would increase. However this will not occur unless the total amount of sodium reabsorbed is also reduced. This was not the case in the present investigation. On the other hand the constant oxygen consumption in the presence of an unchanged absolute amount of sodium reabsorbed may indicate that the basal oxygen consumption of the kidney was also unaffected by the furosemide injection.

The findings are thus all compatible with a normal stoichiometric relation between the oxygen consumption and the sodium reabsorbed. The question whether furosemide interferes with the sodium reabsorption by affecting the permeability of the tubular cells, i.e. by a change without energy cost, as recently claimed by Fülgraff (3) or by a change in the aerobic metabolic processes in the ascending loop of Henle, cannot be answered by the present study.

The ratio between the sodium reabsorption and the oxygen consumption in this group of hypertensive individuals was less than that usually observed in dogs and that reported in a mixed patient group (8). this might indicate a relatively high basal metabolic level in these kidneys. On the other hand the regression coefficients (Fig. 1) were also greater than reported in dogs. The observations are however far too few to permit a definite conclusion.

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PLASMA CORTICOSTEROIDS IN CUSHING'S SYNDROME

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Abstract. Circadian rhythm of plasma corticosteroids and the plasma corticosteroid response during both the 4-hour ACTH test and the 1 mg dexamethasone suppression test (1 mg-DST) have been studied in seven cases of Cushing's syndrome. The early morning baseline values were normal in five out of seven, but elevated in all cases at 10 p.m. The plasma corticosteroid response during the ACTH test was increased in five cases (Morbus Cushing 3, adrenal adenoma 1, ectopic ACTH syndrome 1), normal in one (Morbus Cushing) and subnormal in one (adrenal carcinoma). In contrast to plasma corticosteroid values of less than 6.2 μg/100 ml during 1 mg-DST in patients without Cushing's syndrome no values lower than 8.4 μg/100 ml were obtained on 14 occasions in six cases of Cushing's syndrome. Despite sources of error these procedures, particularly the 1 mg-DST, are valuable in the diagnosis of Cushing's syndrome. A striking agreement between urinary excretion of 17-KGS and plasma corticosteroids was obtained during the DST of Liddle. Greatly elevated baseline values of plasma corticosteroids and subnormal response during the 4-hour ACTH test suggest the presence of extra-pituitary spontaneous Cushing's syndrome.

The most common screening tests for spontaneous Cushing's syndrome are estimations of urinary metabolites of cortisol in basic conditions and during the dexamethasone suppression test (DST) of Liddle (16); furthermore, this DST has been of great value in the aetiological diagnosis before treatment. Usually one measures the urinary 17-ketogenic steroids (17-KGS) or the 17-hydrocorticosteroids (17-OHCS). However, measurement of unconjugated plasma cortisol has some advantages over the urinary assays. Inaccuracies in the collection of urine are avoided, plasma cortisol studies are quicker and are easily carried out.

The first tests, based on plasma cortisol assays, used in the diagnosis of Cushing's syndrome were estimations of basic values and the circadian rhythm (C.R.) of plasma cortisol and the plasma cortisol response during the short-term corticotrophin stimulation test (ACTH test). Recently a simplified DST, evaluated by changes in plasma cortisol, has been introduced as a screening test in Cushing's syndrome (24).

C.R., as evaluated by two daily estimations of plasma cortisol, has been studied in many cases of Cushing's syndrome (9, 11, 1., 23, 29, 31), but there are few reports on C.R. studied by more frequent estimations of plasma cortisol (8, 18, 32). The short-term ACTH test (6, 11, 18, 19, 29, 33) and the simplified DST (24, 26, 31, 35) have been carried out in a few cases of Cushing's syndrome. There are very few reports on simultaneous studies of the C.R. of plasma cortisol and of the response during short-term ACTH test in Cushing's syndrome; no reports have been published on simultaneous studies of C.R. and of plasma cortisol response during short-term ACTH test and during the simplified DST of Nugent et al. (24).

In the present study this has been done in seven cases of spontaneous Cushing's syndrome. In contrast to most of the above mentioned studies plasma cortisol was estimated fluorimetrically (22). C.R. was evaluated by 4–5 daily estimations of plasma corticosteroids. Finally, preliminary experiences with the diagnostic value of plasma corticosteroid determinations during the DST of Liddle (16) are reported.

METHODS

The diagnosis of Cushing's syndrome is based partly on the clinical symptoms and signs and partly on the finding of elevated baseline values of urinary 17-KGS with insufficient suppression of 17-KGS during DST according to Liddle (16).

Determinations of urinary 17-KGS (10) and of urinary 17-ketosteroids (17-KS) (20, 36) are carried out by

Table I. *Clinical signs and symptoms in seven cases of Cushing's syndrome*

M = menopause, GTT = glucose tolerance test, N = normal, A = adrenal adenoma, H = adrenal hyperplasia, C = adrenal carcinoma, = histologically verified, † = verified by clinical and biochemical remission

Case no.	Sex	Born	Height (cm)	B.wt. (kg)	Obesity	Moon face	Hirsutism	Muscular weakness	Menstrual irregularity	Acne	Striae distensae cutis	Buffalo hump
1	♂	1912	174	96		+	−	+		+	+	−
2	♀	1914	158	68	+	−	+	+	M	+	−	−
3	♀	1943	174	59	−	+	+	+	+	+	−	−
4	♀	1905		81	+	+	−	+	M		−	−
5	♀	1916	158	63	−	−	+	+	+	−	−	+
6	♀	1907	164	53	−	−	+	+	M	−	−	−
7	♀	1919	164	99	+	+	+	+	M		−	+

Statens Seruminstitut or by Medicinsk Laboratorium. Baseline values of 17-KGS were evaluated with reference to *Jørgensen* (14) control material, baseline values of 17-KS were evaluated with reference to Statens Seruminstitut control material. A reduction of urinary 17-KGS to values below 4 mg/24 h during DST with 2 mg dexamethasone daily for 2 days is regarded as normal response (5); reduction of urinary 17-KGS to 50% of baseline values during DST with 8 mg dexamethasone daily for 2 days is regarded as positive response (16).

The C.R. of plasma corticosteroids was investigated on heparinized venous blood, sampled at 7 a.m., 9 a.m., 12 noon, 7 p.m. and 10 p.m. A 4-hour ACTH test was carried out as described elsewhere (2): 250 μg β^{1-24} corticotrophin (Synacthen®) was dissolved in 500 ml 0.9% NaCl and infused from 9 a.m. to 1 p.m. Before and during the ACTH test heparinized venous blood was sampled hourly and in the same 24-hour period urinary 17-KGS were determined. Alterations in 17-KGS during ACTH test were evaluated with reference to the control material of Asfeldt and Nielsen (4). A simplified DST (1 mg-DST) was carried out as described earlier (1): on the first day at 8 a.m. plasma corticosteroids were determined and at 11 p.m. 1 mg of dexamethasone was given orally. On the next morning at 8 a.m. the plasma corticosteroids were again determined. In three patients the simplified DST was repeated with 2 mg dexamethasone orally at 11 p.m. During DST according to Liddle (16) the plasma corticosteroids were determined each morning at 8 a.m. in four patients.

Plasma corticosteroids were determined fluorimetrically (22). This method determines 11-hydroxycorticosteroids (cortisol + corticosterone) in plasma.

CASE REPORTS

Case 1

(Hillerød, Med. Dept. F.) Urography and retroperitoneal pneumography showed suspicion of tumour at the site of the left adrenal. Left-sided adrenalectomy was performed. The adrenal was normal but an unusually large quantity of periadrenal fatty tissue was found. Because of no clinical remission, and on the supposition of pituitary-conditioned hypercorticism, he underwent X-ray treatment of the pituitary in Jan.–June 1965. Autopsy in June 1966: the right adrenal was found to be macroscopically normal. On the dorsal site of the right kidney a 1.5 × 1.5 × 1.5 cm tumour was found. Histology: low malignant sarcoma, or polymorph-celled ectopic adrenal adenoma.

Case 2

(Steno Memorial Hospital (S.M.H.)) X-ray treatment of the pituitary in Jan.–Feb. 1966 (3 000 r) and in August 1968 (3 000 r).

Case 3

(S.M.H.) In Feb. 1968 bilateral adrenalectomy was performed (KAS Gentofte, Surg. Dept. H). Weight of right and left adrenal 7 and 9 g, respectively. Histology: pronounced hyperplasia of zona reticularis and zona fasciculata and vacuolization in zona fasciculata.

Case 4

(Blegdamshospitalet, Med. Dept.) X-ray treatment of the pituitary. Post-treatment control not possible.

Case 5

(S.M.H.) In 1961 a 8 × 9 × 10 cm left-sided adrenal tumour was removed (KAS Gentofte, Surg. Dept. H). Histology: carcinoma solidum corticale suprarenale. In 1967 she was admitted to S.M.H. with oedema and hypokalaemic alkalosis. Autopsy in April 1967: metastases in the lungs, on the pleura and on the diaphragm, histologically of the same appearance as the carcinoma found earlier. The right adrenal was atrophic.

Case 6

(S.M.H.) In June 1967 admitted to S.M.H. with hypokalaemic alkalosis. Bilateral adrenalectomy was performed (KAS Gentofte, Surg. Dept. H). Weight of right and left adrenal 30 and 20 g, respectively. Histology:

Blood tension (mmHg)	Diabetic oral GTT	Sella turcica	Osteo-porosis	Visual defect	Cause of hyper corticism
220/140	+	N	+	–	A or H
180/110	+	N	–	–	H †
150/70	+	N	–	–	H
190/90	+	N	–	–	H
155/80	+	N		–	C
190/100	–	N	–	–	H
175/115	+	N	–	–	H

adrenal hyperplasia with metastasis to one adrenal from carcinoma solidum anaplasticum. Post-operatively tumour-like alterations in the right lung were found by X-ray. She died in 1967. Autopsy was not performed.

Case 7

(S.M.H.) In Feb. 1971 bilateral adrenalectomy was performed (Rigshospitalet, Surg. Dept. R). Weight of right and left adrenals 16 and 11 g, respectively. Histology: adrenal hyperplasia.

MATERIAL

The material comprised seven patients with Cushing syndrome (Table I).

Table II shows baseline values of 17-KGS, 17-KS/24 h urine, and the values of 17-KGS during ACTH test and during DST according to Liddle (16). In all cases Cushing's syndrome was biochemically verified by the finding of elevated baseline values of urinary 17-KGS and of the insufficient response during the DST of Liddle (16). In case 4 urinary 17-KGS were not, unfortunately, determined during the low-dose DST. However, as 17-KGS only fell to 9.0 mg/24 h urine during the high-dose DST it is improbable that 17-KGS would have been suppressed to values below 4 mg/24 h urine during low-dose DST.

The diagnosis of pituitary-conditioned hypercorticism (Morbus Cushing) was histologically verified in cases 3 and 7 and verified by clinical and biochemical remission following X-ray treatment in case 2. The presumptive diagnosis of Morbus Cushing in case 4 was based solely on clinical and biochemical findings. In case 5 the cause was histologically verified. In case 6 the histologically verified adrenal metastasis was presumably secondary to the tumour-like alterations in the right lung, demonstrated by X-ray. The diagnosis of ectopic ACTH syndrome is highly probable. The clinical course, the results of DST and the autopsy results in case 1 indicate an adrenal

Table II. *Baseline values of 17 KGS and 17 KS and values of 17 KGS during 4-hour ACTH test and during DST according to Liddle (16)*

Case no.	Time of admission	17-KGS (mg/24 h urine) Baseline values	During ACTH test	DST daily dexamethasone 2 mg	2 mg	8 mg	8 mg	17 KS baseline values (mg/24 h urine)
1	Oct. 64	33.7–25.8	—	34.8	36.4	31.0	26.5	23.2
	Jan. 65	26.3–17.4	—	18.3	14.3	12.3	9.9	11.5
	May 65	12.7	—	19.8	16.4	16.3	15.1	—
2	Dec. 65	34.8–17.4	59.5	—	—	—	—	22.4–10.6
	Jan. 66	30.2–30.9	—	22.4	20.2	12.8	6.0	19.6–18.0
	May 66	26.2	—	—	—	—	—	12.8
	Aug. 66	17.0	—	—	—	—	—	12.5
	Jan. 67	15.8	—	—	—	—	—	16.0
	Sept. 67	16.0	—	—	—	—	—	14.9
	Aug. 68	20.3–16.5	—	15.1	14.4	9.4	3.4	11.4–11.0
	Jan. 69	11.8–10.6	—	—	—	—	—	—
3	Sept. 66	18.1	46.5	29.9	19.2	9.7	4.8	21.1
	Jan. 67	24.2	—	25.7	20.2	10.6	7.3	19.2
	July 67	24.8–17.6	—	—	—	—	—	18.6–15.1
	Feb. 68	20.9	—	—	—	—	—	16.6
	Oct. 68	25.1–22.6	—	—	4.1	—	2.7	19.8–19.8
	Feb. 69	26.4	—	—	—	—	—	21.3
	March 69	0.0	0.0	—	—	—	—	4.0
4	Jan. 67	21.6–15.8	—	—	—	—	9.0	17.[illegible] 14.0
5	Nov. 61	40.8	—	—	—	—	—	44.0
	Dec. 61	33.0	—	—	—	—	—	3.0
	Feb. 67	111.0	88.0	111.0	96.0	61.0	44.7	159.0
6	June 67	116.0–56.3	54.8	117.0	142.0	123.0	108.0	59.5–41.0
7	Aug. 70	27.3–29.4	—	16.1	9.0	3.4	2.1	15.6

Table III. *C.R. of plasma corticosteroids in six patients with Cushing's syndrome*

Case no.	Time of admission	Plasma corticosteroids (µg/100 ml) 7 a.m.	9 m.	12 a.m.	07 p.m.	10 p.m.
1	Oct. 64	24 0	27.0	26 0	26.0	23.0
2	Dec. 65	21 1	—	—	—	24.0
	Jan. 66	24.3	—	21.6	18.8	20.8
	May 66	26.9	20 6	17.6	18.2	15.3
	Aug. 66	24.5	—	20 1	24.5	15 7
	Sept. 67	27 4	—	20.0	25.3	17.5
3	Sept. 66	15.2	17 7	17.5	17.3	17 6
	Jan. 67	15.4	15 4	18.8	16.8	15 7
	July 67	19.3	26.1	22.2	17 7	17.0
	Feb. 68	15.3	15.2	13.3	12.5	13.8
	Feb 69	18.2	—	15.6	13.8	16.9
4	Jan. 67	24.0	24 0	—	15 0	17.0
5	Feb. 67	41.2	43.3	35 4	38.8	37.2
6	June 67	61.0	58.0	57.5	50.8	51.0
Controls Mean ± 2 S.D.	Female (= 10) Male (n = 8)	13 6–35.5 8.5–26.7	6.3–19.9	6.7 16.9	4.5–13 7	1 1 11.5

Published by Asfeldt (2).

adenoma as the more likely cause of the hypercorticism, even though the histological description of the tumour was insufficient. Ectopic ACTH syndrome is improbable as the remaining adrenal was not hyperplastic. An alternath aetiology is hypophyseal-conditioned Cushing syndrome.

RESULTS

The C.R of plasma corticosteroids is seen in Table III. In case 7 the baseline values of plasma ticosteroids at 8 a.m. and 10 p.m. were 19.3 14.5 µg/100 ml, respectively In five patients (cases 1 2 3 4 and 7) normal alues of plasma corticosteroids were found in the early morning. Elevated values were found during the rest of the day with the exception of case 3 however she always had elevated values at 10 p.m. In cases 5 and 6 the values were elevated throughout the whole day On five occasions a considerable fall was found in plasma corticosteroids in the course of the day (cases ., 3 4 and 7)

The results of the ACTH test are shown in Table IV In five patients (cases 1 2, 4 6 and 7) plasma corticosteroids rose greatly to abnormally high values. In case 3 a normal response was found, after total adrenalectomy there was no response during the ACTH test. In case 5 a subnormal increase in plasma corticosteroids was observed during the ACTH test.

The results of DST can be seen in Table V 1 mg DST was carried out several times in cases 2 and 3 and once in four patients. During 1 mg DST a minor fall in plasma corticosteroids was observed several times, but a normal suppression to values below 6 µg/100 ml (1) was never found. The lowest value of plasma corticosteroids during 1 mg-DST was 8 4 µg/100 ml. Even after clinical and biochemical remission of the hypercorticism there was still no response during 1 mg-DST in case 2. During 2 mg DST a further fall in plasma corticosteroids was observed in cases 2, 3 and 7 but only on one occasion to a value below 6 µg/100 ml.

During DST according to Liddle (16) the alterations in plasma corticosteroids principally followed the alterations in urinary 17 KGS In cases ., 3 and 7 with pituitary-conditioned hypercorticism, a certain fall in plasma corticosteroids was observed during low-dose DST but the values did not fall to the levels seen during the 1 mg DST in patients without hypercorticism (1) During high-dose DST plasma corticosteroids were further suppressed in cases 2, 3 and 7 but in case 5 with autonomous hypercorticism, practically no alteration in plasma corticosteroids was observed

DISCUSSION

The diagnostic values of C.R. of plasma cortisol, of the 4-hour ACTH test and of the 1 mg DST

Table IV *Plasma corticosteroids during 4-hour ACTH test in seven cases of Cushing syndrome*

Case no.	Time of admission	Plasma corticosteroids (μg/100 ml) 9 a.m.	10 a.m.	11 a.m.	12 m.	01 p.m.	02 p.m.
1	Oct. 64	27 0	40.0	—	61 0	69 0	74 0
2	Dec. 65	26 6	—	—	—	106 0	102 0
3	Sept. 66	15.8	31.2	38.6	45 8	49.3	54 6
	March 69	0 6	0.6	0.7	0 4	0.6	0 9
4	Jan. 67	19.0	75 0	110 0	112 0	154 0	—
5	Feb. 67	36 0	38 2	38.1	39 4	40.3	39 4
6	June 67	68 1	—	—	—	117.0	127 0
7	Aug. 70	15 4	39 4	49.8	67 4	62 7	57 6
Controls[a] Mean ± 2 S D	Female (= 10)	8 2 24 4	22.5–35 7	25.8–41.9	28.8–46 9	29.8–53 4	32.3–56.7
	Male ($n = 8$)	8.2 22 4	21.5–29 4	27.5–32.9	28.5–36.3	32 4–41 1	30.4–41 7

[a] Published by Asfeldt (2).

depend on the validity of the tests in distinguishing cases with hypercorticism from those without hypercorticism, and on the validity of the tests in the aetiological diagnosis.

Elevated baseline values of plasma cortisol are a characteristic finding in Cushing s syndrome (9 11 12, 23 24 27 29 31 33). However in approximately one-third of the cases normal 8–9 a.m. baseline values of fluorimetrically deter mined plasma corticosteroids ha e been observed (12) In the present study normal early morning baseline values of plasma corticosteroids were found in five of seven cases. Lindsay et al (18) showed that there was no C.R of plasma 17 OHCS in Cushing's syndrome. In 21 cases of Cushing s syndrome Ekman et al. (8) found that

Table V *Plasma corticosteroids during DST according to Liddle (16) and during simplified DST according to Asfeldt (1) in six cases of Cushing s syndrome*

Case no.	Time of admission	Plasma corticosteroids (μg/100 ml) Baseline value	DST Daily dexamethasone 2 mg	2 mg	8 mg	8 mg	Simplified DST Baseline value	After 1 mg	After 2 mg
2	Jan. 66	27.2	20 4	25 0	7.6	9 1	—	—	—
	May 66	—	—	—	—	—	26 9	21.2	—
	Aug. 66	—	—	—	—	—	24 5	18.4	—
	Jan. 67	—	—	—	—	—	20.7	21 4	—
	Sept. 67	—	—	—	—	—	27 4	20 6	19 5
	Aug. 68	17 7	17.3	—	4 9	5.2	—	—	—
	Jan. 69	—	—	—	—	—	20.4	17.9	—
3	Sept. 66	25.8	16.4	16 6	7.6	6 8	18 6	14 6	—
	Jan. 67	15.3	9.2	7.8	5.4	6.4	18.4	15 0	—
	July 67	—	—	—	—	—	26 1	10.7	—
	Feb. 68	—	—	—	—	—	17.2	14 1	7 7
	Feb. 69	—	—	—	—	—	18.2	8 4	5 4
4	Jan. 67	—	—	—	—	—	22 0	18 9	—
5	Feb. 67	37 7	35.2	35.5	36.4	35.8	41.3	40.0	—
6	June 67	—	—	—	—	—	38 0	37.5	—
7	Aug. 70	19 1	8.3	6.7	2.3	2 4	21.9	18.3	11 7

plasma 17-OHCS remains at almost the same level throughout the 24 hours. However in three cases there was a noticeable fall in plasma 17 OHCS in the evening. By fluorimetric determination of plasma corticosteroids every 20 min during a 26-hour period in four cases of Cushing's syndrome Sederberg et al. (32) found elevated evening values in all cases. In the present study the C.R. was investigated 14 times in seven cases of Cushing's syndrome. On five occasions a limited fall in the values was found in the evening. The 10 p.m. baseline values were always elevated. In other investigations, however normal baseline values have been found in the evening in a few cases (9 29). The highest baseline values are most often seen in ectopic ACTH syndrome (11 17) which was also the case in patient no 6 in the present study However an absent C.R has also been found in patients with severe brain damage (7) Furthermore, elevated baseline values are seen during pregnancy and in women using contraceptive pills, but the C.R. is here preserved (2)

As Laidlaw et al. (15) and Hinman et al. (13), during ACTH test, found elevated adrenal response in Morbus Cushing and normal or no adrenal response in adrenal tumour (adenoma, carcinoma) they suggested that the ACTH test is suitable in the aetiological diagnosis of Cushing's syndrome. Exceptions from these rules have been described later in Morbus Cushing (9 16, and in adrenal adenoma (16, 29) No adrenal ... is a characteristic feature in ectopic CTH syndrome (28) but exceptions from this rule have been described (5 25) In Cushing's syndrome, the short-term ACTH test evaluated by plasma cortisol, generally gives the same results as the above mentioned ACTH tests evaluated by the urinary excretion of corticoid metabolites. However in 10–20% of chronically ill patients without Cushing's syndrome an elevated response of plasma 17-OHCS is seen (6 21)

In Morbus Cushing a normal or elevated response of plasma cortisol has been found during the short-term ACTH test (6 29 33) In the present study the response during the 4-hour ACTH test was elevated in three cases and normal in one case of Morbus Cushing.

In adrenal adenoma, elevated (21) normal (23 33) and subnormal (6 33) responses have been described during short-term ACTH test. In case 1 with presumed adrenal adenoma an increased response of plasma corticosteroids was found during the ACTH test.

In case 5 with adrenal carcinoma, almost no increase in plasma corticosteroids was observed during the ACTH test. This finding is characteristic in adrenal carcinoma with hypercorticism (6, 18 19) but a doubling of plasma 17-OHCS has nevertheless been described in a few cases (19)

In ectopic ACTH syndrome subnormal responses of plasma cortisol have been seen and are a characteristic feature during the short term ACTH test (11 28) In case 6 with probable ectopic ACTH syndrome an increased plasma corticosteroid response was found during the ACTH test.

During the 1 mg-DST Nugent et al (24) found values of plasma 17-OHCS < 11 µg/100 ml in 139 subjects without Cushing's syndrome and plasma 17-OHCS values > 20 µg/100 ml in nine cases of Cushing's syndrome. Later investigations have shown that a suppression of plasma 17-OHCS (26) of fluorimetrically determined plasma corticosteroids (1 31) or of plasma cortisol, determined by the double isotope-derivative method (35) to values lower than 5–6 µg/100 ml excludes with great certainty the diagnosis of Cushing's syndrome

In ten cases of Cushing's syndrome (Morbus Cushing 7 adrenal adenoma 1 adrenal carcinoma 2) Tucci et al. (35) found values of plasma cortisol > 8 µg/100 ml during the 1 mg-DST in one of these cases, a patient with mild, early Cushing's syndrome, a normal response was obtained in the first of three 1 mg DST In 17 cases (Morbus Cushing 10 adrenal adenoma 5 adrenal carcinoma 2) Pavlatos et al. (26) found no values of plasma 17-OHCS below 13 µg/100 ml during the 1 mg-DST In two cases of Cushing's syndrome studied by Sawin et al. (31) plasma corticosteroids were 8 7 and 9 5 µg/100 ml during 1 mg DST In two obese patients Sawin (30) obtained a normal response during the 1 mg DST although the low-dose DST of Liddle (16) indicated Cushing's syndrome. This last result was suggested to reflect a high cortisol secretion rate in obese subjects.

In the present study the 1 mg-DST was carried out 14 times in six cases of Cushing's syndrome. Plasma corticosteroids were no lower than 8 4

μg/100 ml during the test. However in one case (no. 3) plasma corticosteroids were suppressed to 5.4 μg/100 ml during 2 mg-DST. Insufficient suppression of plasma corticosteroids during 1 mg-DST has also been described in patients without Cushing's syndrome, i.e. in women using contraceptive pills, in pituitary adenoma, in anorexia nervosa, in patients receiving phenytoin or primidone (1, 3) and in acute illness (24).

During the DST according to Liddle (16) the daily 8 a.m. plasma corticosteroids were estimated 6 times in four cases. A striking agreement between alterations in urinary 17-KGS and plasma corticosteroids was obtained. Further investigations will show the degree to which determination of plasma corticosteroids can replace urinary 17-KGS determination in the evaluation of the DST of Liddle (16).

Thus an absent C.R., elevated baseline values of plasma cortisol in the evening, an increased plasma cortisol response during the 4-hour ACTH test and an insufficient suppression of plasma cortisol during the 1 mg-DST strongly indicate Cushing's syndrome. In a review of earlier reports on Cushing's syndrome (21) it was found that C.R. of plasma cortisol is absent in about 87% of all cases, and that an abnormally high increase in plasma cortisol during short-term ACTH test is to be seen in about 88% of all cases. In contrast, insufficient suppression of plasma cortisol during 1 mg-DST has been found in all cases of clinically and biochemically manifest cases of Cushing's syndrome.

Therefore the 1 mg-DST is superior to the C.R. and the 4-hour ACTH test. In the diagnosis of hypercorticism the 1 mg-DST presumably is as valid as the low-dose DST of Liddle (16), perhaps even better, as Streeten et al. (34) found a normal suppressibility of urinary 17-OHCS during the low-dose DST of Liddle (16) in three out of 17 cases of Cushing's syndrome. The present results support an earlier supposition (33) that greatly elevated baseline values of plasma cortisol and abnormal response during the 4-hour ACTH test suggest the presence of extra-pituitary Cushing's syndrome.

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GENERALIZED SCLERODERMA

Report on a Case Associated with Acute Nephropathy, Haemolytic Anaemia and Malignant Ovarian Tumour

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Abstract. A 64-year-old female farmhand developed generalized scleroderma, causing death nine months after the first manifestation of symptoms. The cause of death was acute sclerodermic nephropathy with characteristic clinical changes in kidneys and skin. The BP remained normal. A concomitant severe haemolytic anaemia is described, and the importance of an associated serous cystadenocarcinoma of the ovaries is discussed.

Generalized scleroderma (g.s.) is a rare disorder involving thickening of the skin and visceral lesions, particularly in the gastrointestinal tract, the lungs and the kidneys. Histologically an increased amount of dense collagen in the connective tissue and typical vascular lesions will be found.

The aetiology and pathogenesis are unknown and were recently discussed by Rodnan (13). Infections, chemical intoxication, endocrinous and immunological disturbances have been suggested as possible contributing factors. Also the association between carcinomas and various disorders of the connective tissue—including scleroderma—has been discussed (11, 15).

In most cases the disease runs a protracted course (9) and very few cases of acute nephropathy associated with this disease have been reported by Danish investigators (2, 8). The following is a report on a recent case presenting several diagnostic and therapeutical problems.

CASE REPORT

The patient was an unmarried 65-year-old female farmhand. There was no family history of disorders of the kidneys, the blood or the connective tissue. The menopause had occurred at the age of 48, and she had had no gynaecological complaints.

During the six months prior to admission she had complained of increasing fatigue and loss of appetite and there had been a 10 kg weight loss. Concomitantly she noticed increasing stiffness, tenderness and pain in the finger-joints with recurrent ulceration of the finger-tips and thickening and stiffness of the skin of the face (particularly around the mouth) and of the limbs. For the last two months she had received Ronicol® and indometacine (Indocid®) without effect. On admission her general condition was good. Typical sclerodermic changes were found in the face and on the limbs. The diagnosis was verified by skin biopsy (see below). BP 150/60 mmHg. ECG and chest X-ray were normal. Gastroscopy revealed atrophy of the mucous membrane and biopsy specimens from the oesophagus and the small intestine showed moderate fibrosis of the submucosa. There was free acid in the stomach. Rectoscopy showed normal conditions. X-ray of the gastrointestinal tract revealed gastroduodenitis and delayed passage. X-ray of the finger-joints showed arthrosis.

Laboratory data on admission

Nephrological findings. Serum creatinine 0.9 mg/100 ml. Also the blood urea and serum electrolytes were within normal range. Microscopy of the urine was normal, and there was no glucosuria, proteinuria or haematuria. Urinary specific gravity was 1.010 with a 24-hour output of 900 ml.

Haematological findings. Hb 9.8 g/100 ml, ESR 31 mm/h, mean corpuscular volume 116 μm^3, mean corpuscular Hb concentration 31 g/100 ml. WBC, differential count and platelet count were normal. Reticulocytes 6.8%, haptoglobin 6 mg/100 ml. Coombs' direct test was negative and the osmotic resistance of the erythrocytes was normal. No leucocyte antibodies in serum. Serum iron and transferrin were normal. Serum vitamin B_{12} 100 pg/ml. Serum folic acid 3 µg/l. Absorption tolerance test revealed vitamin B_{1} deficiency. Schilling test 13%. The sternal bone marrow was hyperplastic with vivid normoblastic erythropoiesis.

Additional laboratory data. Agglutination consumption

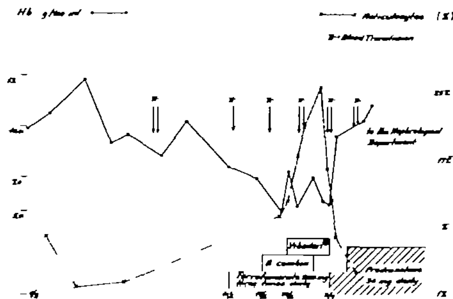


Fig. 1 Anaemia in relation to the treatment. Abscissa: time. Ordinate: (left) Hb, (right) reticulocytes.

test (AGCT) + + + whereas no antinuclear or LE factors were found in the serum. RA test, Rose Waaler test and Wassermann reaction were negative. Serum electrophoresis was normal. Serum LDH was 310 U/l (normal range 65 to 135 U), whereas alkaline phosphatase, GOT and bilirubin were normal. Antistreptolysin titre, antistreptococcic hyaluronidase and thyroid function were also normal.



Fig. 2 Skin with typical sclerodermic lesions—thickened dermis and deeply located atrophic sweat glands. × 35

Course of disease

During the first two weeks after admission the complaints from the joints abated transitorily and during the same period the Hb value increased (12.8 g/100 ml) and the reticulocyte count fell to normal range (1.1%) before any specific therapy had been instituted (Fig. 1). Subsequently the patient developed increasing anaemia with reticulocytosis, increasing ESR and LDH, slight hepatomegaly with concomitant increase in serum bilirubin to 2.1 mg/100 ml. Repeated blood transfusions became necessary. During treatment with B-combin injections, total 24 ml, and hydroxocobemine (Vibeden®), total 4 ml, pronounced reticulocytosis developed, but the Hb value did not increase until prednisolone therapy was instituted.

After 38 days in hospital she developed proteinuria with 0.7–1.5 g protein/day in the urine. Ten days later the creatinine clearance was 49% and the serum creatinine 1.4 mg/100 ml (normal value below 1.3 mg/100 ml). After another three weeks the serum creatinine had increased to 7.3 mg/100 ml, serum potassium to 6.7 mEq/l and serum urea to 354 mg/100 ml. The BP was constantly normal, ranging from 150/90 to 160/90 mmHg.

The patient was then transferred to the Nephrological Department Y for peritoneal dialysis and clarification of the renal disorder. Direct pyelography revealed normal excretory ducts, and renal biopsy showed irreversible lesions (see below). Consequently continued dialysis was considered not to be indicated. The patient was referred back to our department for continued treatment with prednisolone, 15 mg, furosemide (Lasix®), 480 mg, Resonium®, 30 mg, and nortestosterone (Durabolin®), 50 mg daily and parenteral fluid therapy. She died in uraemic coma about 12 weeks after admission.



Fig. 3. Interlobular arteries. A pronounced concentric narrowing of the lumen is seen, caused by heavily thickened intima across which normal media is seen. The intima has an abundant pale mucoid degenerated stroma with fusiform or star-shaped cells. 60.

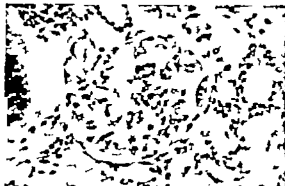


Fig. 4. Arteriolar necrosis in an afferent vessel, extending into the glomerular tuft. The thickening of the basement membrane is also distinctly seen. 40.

Pathological anatomy

Biopsies from the skin revealed thinning and flattening of the epidermis with pronounced basal pigmentation and heavily thickened dermis consisting of connective tissue, poor in cells and with large septa extending down into the subcutis. N sebaceous glands and only few deeply located and atrophic sweat glands were seen (Fig 7). Apart from single perivascular round-cell infiltrate, no vascular lesions ere revealed. The surgical renal biopsy specimen showed changes in all glomeruli, hich ere moderately rich in cells and empty of blood. A few synechiae ere seen, but no crescents. The basement membrane was moderately thickened (Fig. 4), and in som areas wire-like lesions were formed N hyaline thrombi or haemotoxylin bodies were seen. In several glomeruli fibrinoid necroses were found, with partial granulocyte infiltration of the capillary tuft (Fig. 4). The large vessels were normal, hereas in all the inter lobular—and smaller—arteries pronounced concentric narrowing of the lumen was seen, caused by heavily thickened intima across which normal media seemed to be stretched. The intima consisted of fusiform or star-shaped cells situated in an abundant pale mucoid degenerated stroma (Fig. 3). Those vessels contained few segmental necroses. The lumen of several of the afferent vessels as slightly narrowed, and in some of them fibrinoid necroses were seen, often extending into the capillary network. The tubular lesions were moderate, only few of the proximal tubules being dilated with flattening of the epithelium and containing eosinophilic sediments. In the cortex there were few micro-infarctions, involving one or tw glomeruli and their tubules (Fig. 5). There ere moderate interstitial fibrosis and oedema. The juxtaglomerular apparatus was normal. No perivascular inflammatory infiltrates were found.

Autopsy revealed cachectic oman with typical sclerodermic skin lesions. There were ascites, hydrothorax and slight oedema. The heart was normal, apart from recent (uraemic) pericarditis. The surface of the kidneys was smooth, mottled, with normal parenchymal configuration. Each kidney eighed 120 g. A bil teral ovarian cancer with peritoneal carcinoma and metastas to the para-aortic and mesenteric lymph nodes er f nd.

The sternal bone marrow as slightly hyperplastic normal content of iron and increased mobias erythropoiesis (between 25 and 35 at repea ed cou

Specimens from skin and kidney presented the le n described in the foregoing. Sections from the to g e *the oesophagus, the small intestine, the muscles,* the endocardium and the myocardium showed incr sed fibrosis, hereas no such changes ere found bu lungs. There as pronounced haemosiderosis in the and spleen. Apart from those found in the kidney no vascular lesions or vasculitis ere revealed. Ther er serous cystadenocarcinomas of the ovaries ith merous metastases, some of them visible only under the microscope, in the lungs, the liver and the lymph nodes.

DISCUSSION

The clinical picture in our patient was charac teristic of g.s. and the diagnosis was erified by biopsies obtained before and after death. In pa-

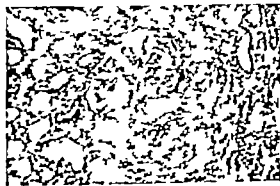


Fig. 5. Micro-infarction in kidney. 4.

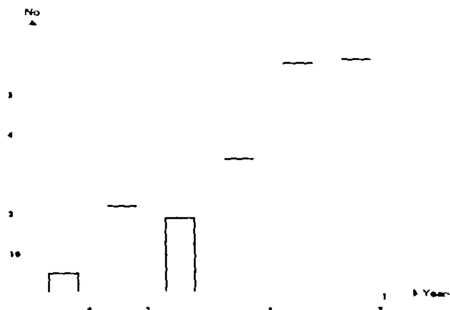


Fig. 1 Morbidity in azotemia correlated to age in 1966–68 in Göteborg.

197 case records. The relevant diagnoses have been checked and in case of death the post mortem findings have been studied. As the investigation extended over a period of three years, some of the cases of azotemic morbidity were also found to be included in the figures of uremic mortality.

Uremic mortality necessitated closer study of 148 case records. The so-called calculated mortality comprised persons who actually died in uremia plus patients who were actively treated with dialysis and/or renal transplantation.

RESULTS

Morbidity

The total morbidity in azotemia, 197 cases, in correlation to age distribution is shown in Fig. 1 from which it is seen that the age groups 55–65 and 66–75 each represent 30% of the total morbidity. There is a tendency to decreasing morbidity from 1966 to 1968. Table I shows the number of cases correlated to the population of Göteborg.

The most frequent causes of azotemia were pyelonephritis, glomerulonephritis and diabetes in the ages between 16–65 years (Table II). The pyelonephritic group showed a decreasing tendency of azotemia of 4.4, 3.6 and 3.4 cases/100 000 inhabitants during 1966, 1967 and 1968 respectively (Table II). The decrease was also seen in the diabetic azotemia. Three cases of diabetes and urinary tract infection have been registered as diabetic nephropathy according to our evaluation of the main cause of renal insufficiency from the case records.

Table I. *Morbidity in azotemia (serum creatinine > 5 mg/100 ml) in 1966–68 correlated to different age groups and to the population of Göteborg*

	Age group 16–75		Age group 16–65	
	No. of cases	Cases/100 000 inhab	No. of cases	Cases/100 000 inhab.
1966	72	16.2	51	11.5
1967	67	15.1	48	10.7
1968	58	13.1	39	8.8
Total no.	197		138	
$\bar{M}/y$	65.6		46.0	

Table II. *Morbidity in azotemia in 1966–68 in age group 16–65 correlated to the population of Göteborg*

	1966	1967	1968	Total	No./y and 100 000 inhab
Pyelonephritis	19	16	15	50	3.8
Glomerulonephritis	12	12	10	34	2.6
Diabetes mellitus	9	7	4	20	1.5
Ren cysticus	3	2	3	8	0.6
Lupus erythem. diss	1	1	2	4	0.3
Nephrosclerosis hypertonia	2	2	—	4	0.3
Malignant diseases	2	2	3	7	0.5
Other diseases	3	6	2	11	0.8

The seven cases of malignant diseases comprise four cases of tumour of the urinary bladder two of multiple myelomatosis and one case of lymphosarcoma.

The eleven cases in the group other diseases include five cases of tubular necrosis, two of Wegener's granulomatosis, two of renal amyloidosis, one of epidemic nephropathy and one of hepato-renal syndrome.

As could be expected (3 7) the females predominate in the pyelonephritic group, with a ratio of 37 females to 13 males. The reverse is true of the glomerulonephritic group, with a ratio of 11 females to 23 males. The same ratio is also found in the diabetic group with 7 females to 13 males.

Mortality

A total of 71 patients died in uremia in the age group 16–65 during the investigation period. During the same period 45 patients were actively treated with dialysis and/or transplantation. Adding these figures gives the so-called calculated mortality of 116. Correlated to the population there is an obvious decrease in the ages between 16–65 years (Table III) from 1966 to 1968.

The dominating groups are pyelonephritis and glomerulonephritis (Table IV). In glomerulonephritis the highest frequency of death in uremia is in the age group 45–55 (Fig. 2). In the pyelonephritic group the highest frequency is found in the age group 56–65 However the number of cases is relatively small.

The nine cases of other diseases (Table IV) at the age of 16–65 years include five cases of hypertension with nephrosclerosis. Included are also two cases of amyloidosis, one of Wegener's granulomatosis and one with malignant tumour of the urinary bladder with metastases. The decreasing tendency of morbidity in pyelonephritic azotemia is also found to be true of pyelonephritic mortality The calculated mortality in pyelonephritis correlated to the population figures is 4 7 3 4 and 3.2 cases/100 000 inhabitants during 1966, 1967 and 1968 respectively The same pattern of female preponderance in the pyelonephritic group among patients with azotemia is repeated in the mortality figures (37/13 = ♀/♂) The reverse is found in the glomerulonephritic (9/17 = ♀/♂) and the diabetic group (3/14 = ♀/♂).

Table III. *Mortality in uremia in 1966–68 in age group 16–65 correlated to the population of Göteborg*

	Dead without active therapy	Actively treated	Calculated mortality	No. of inhab.	Calculated mortality/ 100 000 inhab.
1966	26	20	46	443.292	10.4
1967	22	15	37	445.408	8.3
1968	23	10	33	444 131	7 4

DISCUSSION

Pyelonephritis

The present investigation shows a decrease of uremic mortality and a decrease of morbidity in azotemia in spite of the relatively short investigation period of three years. The decrease of mortality in uremia is mainly seen in the pyelonephritic group. In an earlier investigation (9) the uremic mortality in pyelonephritis in the ages between 30–60 years decreased from 7.9 to 7 4 cases/year and 100 000 inhabitants from the period 1956–60 to the period 1961–65 In the present investigation the pyelonephritic mortality is 2.8 cases/year and 100 000 inhabitants if the same age groups are used. The main explanation is probably a diminishing analgesic abuse. This was present in the case histories of 70% of the patients in the pyelonephritic group of uremic mortality during 1966 (age limits 16–65) and in only 47% of the case histories from 1968. The effect of a law from 1961 when phenacetin-containing drugs (4 8) were restricted to pre

Table IV *Original diagnoses and calculated mortality in uremia in 1966–68 in Göteborg*

	Age group	
	16–65 (a)	16–75 (n)
Pyelonephritis	50	68
Glomerulonephritis	26	30
Diabetes mellitus	17	20
Ren cysticus	9	11
Lupus erythematodes diss.	5	5
Other diseases	9	14
Total no.	116	148
$\bar{M}$ y	38.7	49 3

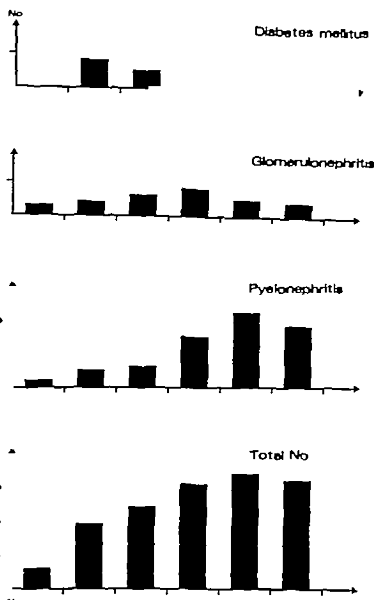


Fig 2 Calculated mortality in uremia correlated to age in 1966-68 in Goteborg.

scription, was already seen in 1964 and 1965 (4) The diminishing number of new cases of pyelonephritis could at that time exclusively be ascribed to a decrease of analgesic abuse, as the incidence of pyelonephritis in non-abusers was unchanged. The present investigation underlines this trend still further but the decrease of uremic mortality in pyelonephritis is even greater than could be explained from the decrease of analgesic abuse.

The increasing awareness of asymptomatic, persisting bacteriuria, the general attention to urinary infections and the use of the proper antibiotic for an adequate period (5 15) are probably contributory factors to the diminishing figures of uremic mortality

Careful management of pyelonephritis will also be responsible for a shift of the mortality figures to older age groups. In this investigation the

number of azotemic patients represents partly a more recent group of pyelonephritic patients than the number of deaths in uremia. Among the azotemic patients the percentage of analgesic abusers is still decreasing. Of the patients registered during 1966 55% had a history of abuse of phenacetin-containing drugs, while only 38% registered during 1968 had a similar history.

Glomerulonephritis

International analyses of mortality statistics of acute and chronic glomerulonephritis generally show a decrease in death rate. These analyses are based upon registered main causes of death, but the reported main cause of death might change with the diagnostic basis, e.g. whether a patient is dying from cardiovascular disease with chronic nephritis or the reverse. The decrease in the mortality figures for glomerulonephritis is still obvious in those registrations where cases of glomerulonephritis are counted if reported either as the primary or the secondary cause of death (10). In an earlier study on mortality in chronic uremia in Göteborg (9) the death rate due to chronic glomerulonephritis was 3.1, 2.8 and 2.7 cases/year and 100 000 inhabitants during three consecutive observation periods during 1950–65. In the same age groups we found a mean value of 1.8 cases/year and 100 000 inhabitants during 1966, 1967 and 1968. The explanation of this can only be speculative at the moment. Perhaps a change in the pathogenetic mechanisms of glomerulonephritis is taking place. The early and adequate treatment of hypertension may be an important factor. The increased use of immunosuppressive drugs may also influence the course of the disease (3, 11). There is, however, still insufficient evidence to show that immunosuppressive treatment really affects the natural course of the disease (2, 6).

Pyelonephritis has a relatively decreasing importance when the relations of uremic mortality and azotemic morbidity between pyelonephritis, glomerulonephritis, polycystic kidneys and diabetes (age group 16–65) are compared. The continuous alterations of the incidence of renal insufficiency illustrate an urgent need for a continuous and thorough registration of patients with renal insufficiency as a basis for calculation of therapeutical resources.

Active treatment of uremia

The low figures of patients actively treated with dialysis or renal transplantation (Table III) are remarkable, as active treatment of uremia has been a serious aim in Göteborg since 1965. The percentage of actively treated patients apparently seems to decrease during the investigation period. The case records of the patients who died in uremia were scrutinized for the possibility that these patients received active treatment according to current criteria. On average only 11 cases each year were not suitable for active treatment and another 2 cases a year were doubtful in this respect. About half of the patients who died in uremia were suitable for active treatment but could not receive it for different reasons.

There are few comparable reports in the literature concerning the incidence of uremia and the need for hemodialysis and renal transplantation. The methods of collecting statistics differ, as do the indications for selecting patients suitable for active treatment. In 1964 Morrin (12) estimated in the area of south-eastern Ontario a prevalence rate of chronic uremia of 2.2 to 3.3 cases/100 000 inhabitants with an upper age limit of 50 years. This report was based on a questionnaire to physicians, but only 33% of the questioned physicians replied. Using hospital records and statistics of mortality v. Watschinger (14) estimated the need for dialysis and renal transplantation as 1.8 cases a year and correlated to 100 000 inhabitants in Upper Austria (1966) using the age limits 15–50 years. De Wardener (13) estimated the need for active treatment in the United Kingdom to be 4 cases/year and correlated to 100 000 inhabitants when analysing mortality statistics (1962) with the age limits 16–55 years. With an upper age limit of 60 years Branch et al. (7) estimated in the same country the need for dialysis and renal transplantation to be 3.9 cases a year correlated to the population (1966–68). Alwall (1) could not find any arbitrary age to be a justifiable obstacle to treatment, and he estimated from an investigation in the south of Sweden a need for hemodialysis of at least 7.5 new cases/year and 100 000 inhabitants (1960–64).

The present investigation revealed 5.4 cases suitable for active treatment/year and 100 000 inhabitants, using age limits 16–60 years. If the upper age limit is raised to 65 years, the need for active treatment rises to 6.2 cases.

CONCLUSION

The investigation of the morbidity in azotemia in Göteborg in 1966–68 resulted in an incidence of 10.3 cases/year correlated to the population and to the age group 16–65 A decrease in morbidity from 11.5 in 1966 to 8.8 in 1968 was registered.

The rate of uremic calculated mortality was 8.7 cases/year correlated to the population and to the age group 16–65 This figure represents a decrease compared to an earlier investigation in Göteborg. The decrease continues during the 3-year investigation period 1966–68 from 10.4 to 7 4

According to current criteria for active treatment of uremia with dialysis or renal transplantation 68% of the uremic patients included in the calculated mortality were suitable for active treatment, but only 39% received this treatment.

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HYPERLIPOPROTEINEMIA IN PATIENTS WITH CHRONIC RENAL FAILURE

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Abstract. Concentrations of triglycerides and cholesterol in serum and the distribution of plasma lipoprotein fractions have been studied in 25 patients with chronic non-nephrotic renal failure. Hyperlipoproteinemia was demonstrated in 17 of them (type II 1 case, type III 1 case, type IV 13 cases, type V 2 cases). None of the recognized causes of secondary hyperlipoproteinemia appeared to be responsible for this high incidence. In 19 patients the i.v. glucose tolerance test showed abnormal responses. For patients with 24-hour urinary losses of protein exceeding 3.5 g a statistically significant (inter-patient) correlation was found between the concentration of triglyceride in serum and the rate of renal protein excretion. Including patients with milder degrees of proteinuria this relationship was obscured, and no further correlation could be established between serum lipid values and proteinuria, renal function or glucose tolerance.

In recent years patients with chronic renal failure without nephrotic features have been shown to have serum triglyceride levels higher than those of normal controls (1). Eventually this observation may prove to have therapeutic implications.

We have investigated the incidence of hyperlipoproteinemia in such patients and classified the lipid abnormality according to the criteria of Fredrickson et al. (6). Such classification invites an even more differentiated approach to the evaluation of therapeutic measures aiming at correcting the abnormality.

METHODS

Blood Hb concentration was determined by the cyanmethemoglobin method. Total proteins in serum were determined refractometrically, protein fractions being separated by electrophoresis on cellulose acetate. Proteins in urine were determined quantitatively by precipitation analysis. Serum and urine creatinine concentrations were determined with picric acid in an alkaline medium, using the Technicon autoanalyzer. Endogenous 24-hour creatinine clearance was determined as an average of three consecutive daily values. Serum urea concentration was measured colorimetrically on the autoanalyzer. Serum uric acid concentration was determined enzymatically by the uricase method. Serum triglyceride was determined as described by Eggstein and Kreutz (5), and serum cholesterol as described by Huang et al. (10). Normal values for serum cholesterol and serum triglycerides are presented in Table I. Plasma lipoprotein fractions were separated by paper electrophoresis as specified by Hatch and Lees (9). In all cases the lipid status was assessed following a 12-hour period of fasting. Blood glucose concentration was measured by a reduction method using the autoanalyzer.

Studies of liver function included evaluation of serum alkaline aminotransferase activity, prothrombin time, serum thymol test, and the serum albumin globulin ratio by electrophoresis. Glucose tolerance was assessed by an intravenous glucose load as described by Lundbæk (13). All samples for lipid status and supplementary studies were drawn simultaneously.

Patients with hyperlipoproteinemia were classified according to the five types defined by Fredrickson et al. (6) on the basis of serum cholesterol and triglyceride levels and the serum lipoprotein pattern: type I represents hypertriglyceridemia, normal serum cholesterol concentration and increased chylomicrons; type II includes normal or slightly elevated serum triglyceride levels, increased serum cholesterol concentration and an accentuated β-lipoprotein band; type III includes hypertriglyceridemia and hypercholesterolemia and also a "broad β-lipoprotein band"; type IV consists of hypertriglyceridemia, normal or slightly elevated serum cholesterol concentration and a pre-β lipoprotein band, and type V of hypertriglyceridemia, normal or increased serum cholesterol concentration and chylomicrons as well as a pre-β-lipoprotein band as found by electrophoresis.

MATERIAL

The material consists of 25 patients and comprises all patients admitted during the study (15.5.1969–15.3.1970)

Table I. *Serum total cholesterol and serum triglycerides in healthy persons (4) Values are given within the 95% range (mean ± 2 S.D)*

	Male		Female	
Age (y)	Cholesterol (mg/100 ml)	Triglycerides (mg/100 ml)	Cholesterol (mg/100 ml)	Triglycerides (mg/100 ml)
<29	140–261	43–175	121 223	45–137
30–39	91 313	49–225	153–285	40–152
40–49	167 297	65–210	134–315	41 181
>50	178–291	45–226	191 294	53–139

who fulfilled the criteria given below Data on age, sex, diagnosis and the duration of renal failure are given in Table II.

I all patients the diagnosis f chronic renal failure was supported by studies of serum creatinine and serum urea concentrations as well as the creatinine clearance. Patients with nephrotic syndrome (protein excretion in urine >3.5 g/day serum albumin concentration <.5 g/100 ml, total proteins in serum <6.0 g/100 ml, and oedema (8)) as well as patients with diabetes mellitus or thyroid disease were excluded from the study

All patients were on their usual diet, and none were given low protein–high caloric diets. The condition of some patients suggested prolonged malnutrition. The body weight of 13 patients was normal, whereas 11 were underweight (nos. 9 12 and 22 more than 20%) Patient 24 was obese (24% above normal)

Sixteen patients had arterial hypertension (diastolic BP > 110 mmHg and/or hypertensive retinal changes). None of the patients was grossly overhydrated or treated with steroid or androgen hormones. Six patients had bacturia, none had bacteremia.

Seven months prior to enrolment in the study patient 3 had single peritoneal dialysis. None of the re ng patients had been dialysed. In 13 patients the diagnosis of chronic renal failure was established by renal biopsy and papillary ejection had been demonstrated in two patients. In the remaining patients the diagnosis was based on case histories, signs and symptoms, laboratory tests, and X-ray examinations. Renal biopsy was not indicated in nine patients with obvious chronic pyelonephritis, contra-indicated in one patient with glomerulonephritis and severe arterial hypertension and in two patients with only one kidney Cases of chronic pyelonephritis due to bacterial infection and to abuse of analgetics were classified together

RESULTS

The results are presented in Table II. Eight patients had a normal serum lipid status, whereas 17 patients had hyperlipoproteinemia, 13 type IV two type V one type II and one type III. Therefore 15 patients showed hypertriglyceridemia and hyper-pre-β-lipoproteinemia. It is remarkable that the two brothers (patients 17 and 18) with the same renal disease, have different types of hyper lipoproteinemia. Comparison with the electrophoretic pattern of normal serum failed to demonstrate the presence of a reduced α-lipoprotein fraction in serum from any patient.

For statistical purposes the material was subdivided as follows. 1) the total material (25 patients) 2) eight patients with normal lipid values, and 3) 17 patients with hyperlipoproteinemia.

For each group the lipid values were correlated to the variables listed in the sequel. Also we have examined the inter-group differences by variance analysis (F-test) the Student s t-test, and the χ^2-test. No correlation was demonstrated between lipid values and renal function as expressed by serum concentrations of creatinine and urea or by the creatinine clearance just as there appeared to be no significant difference in renal function between the patients of groups 2 and 3

There was no difference in the number of patients with hypertension in groups consisting of patients with mild, moderate or severe impairment of renal function. Furthermore, no significant correlation was found between the serum lipid values and rates of urinary loss of protein apart from a significant positive correlation between serum triglyceride levels and the 24-hour protein excretion in patients with an excretion of more than 3 5 g/day (6 patients) ($r = 0.86$, $0\,025 < p < 0\,05$) If the correlation analysis is extended to include patients with a protein excretion of more than 3 g/day (9 patients) the statistical significance is lost. There was no significant difference with respect to rates of protein excretion between patients of groups 2 and 3 Finally there was no significant correlation between serum albumin levels and rates of protein excretion and no significant difference

Table II *Data on the patients included in the study*

Triglyc. = serum triglycerides, Chol. = total serum cholesterol, Type = type of hyperlipoproteinemia (5), (N = normal), K = result of i.v. glucose tolerance test (12), Creat. = serum creatinine, C_{creat} endogenous 24-hour creatinine clearance, Urea = serum urea

Pat. no.	Sex	Age (y)	Duration (y)	Diagnosis	Type	Triglyc. (mg/100 ml)	Chol. (mg/100 ml)	K	Creat. (mg/100 ml)	C_{creat} (ml/min)	Urea (mg/100 ml)	Protein excretion (g/24 h)	Hb (g/100 ml)
1	♀	57	8	Chronic pyelonephritis, necrotizing papillitis	IV	233	157	1.44	2.1	27	38	0	12.7
2	♂	58	4	Gout kidney	V	892	297	0.56	4.6	29	72	3	9.4
3	♀	53	8	Chronic pyelonephritis	IV	179	201	1.09	7.3	4	170	0	11.1
4	♂	23	7	Chronic pyelonephritis	IV	176	170	0.85	18.0	3	351	4	7.8
5	♀	61	7	Chronic pyelonephritis	IV	142	170	0.53	3.0	17	91	1	10.2
6	♂	43	2/12	Chronic pyelonephritis, necrotizing papillitis	IV	246	203	1.12	5.5	16	106	1	12.5
7	♂	24	10	Congenital malformations	IV	281	258	1.02	4.7	21	104	3	12.6
8	♀	50	6	Chronic pyelonephritis	IV	162	207	0.53	5.4	7	138	2	11.8
9	♀	33	3	Chronic pyelonephritis	IV	335	195	1.65	5.7	9	225	2	11.9
10	♀	40	9	Chronic pyelonephritis	N	123	193	1.13	5.7	13	124	2	8.1
11	♀	18	2	Chronic pyelonephritis	III	164	268	1.38	1.9	34	49	5	12.7
12	♀	73	6/12	Chronic pyelonephritis	N	89	189	0.43	2.6	24	98	0	13.9
13	♀	37	1	Chronic pyelonephritis	IV	372	268	0.56	6.9	9	128	1	10.5
14	♀	68	8	Chronic pyelonephritis	IV	296	242	1.27	3.7	11	79	2	7.7
15	♂	54	1	Chronic glomerulonephritis	N	179	198	0.76	12.3	6	246	2	7.6
16	♂	39	11	Renes cystica	V	712	367	—	13.2	5	175	1	8.9
17	♂	30	1	Hereditary nephropathy	II	108	519	1.80	4.6	15	92	5	12.2
18	♂	31	11	Hereditary nephropathy	IV	542	271	0.65	13.5	6	191	7	9.4
19	♀	50	2	Chronic pyelonephritis, stones	IV	264	289	0.66	1.5	36	44	0	16.7
20	♀	35	7	Chronic pyelonephritis	N	143	213	1.02	12.3	4	184	4	9.2
21	♀	51	12	Chronic pyelonephritis	N	87	272	0.39	5.9	7	66	8	10.5
22	♂	16	16	Chronic glomerulonephritis	N	172	177	1.15	3.3	13	136	1	12.4
23	♀	47	1/12	Chronic pyelonephritis	N	171	208	0.79	3.4	15	103	1	11.4
24	♂	43	4/12	Chronic glomerulonephritis	N	147	274	0.40	12.9	6	219	5	7.7
25	♀	38	11/12	Chronic pyelonephritis	IV	153	238	0.69	11.1	4	187	3	8.4

in serum albumin levels between the three groups of patients.

As shown in Table II five patients had a normal i.v glucose tolerance test, 12 had diabetic K values, and seven had intermediate K-values. Patients 3 and 12 had fasting blood sugar concentrations of 126 and 125 mg/100 ml, respectively Patients 17 and 18 (brothers) had slight glucosuria. None of the other patients had elevated blood sugar concentrations while fasting or glucosuria. No correlation was established between lipid values and K-values, and no difference was demonstrated between K values for the three groups of patients. Furthermore, there appeared to be no difference in the number of patients with hyperlipoproteinemia or in the number of patients with a normal serum lipid status among patients with diabetic, borderline or normal glucose tolerances.

If two of the liver function tests described in the foregoing were abnormal, the patient was considered to have slight liver impairment, with three or more abnormal tests the liver impairment was considered pronounced. According to these criteria one patient (no. 12, normal lipid values) had pronounced hepatic involvement. Patients 11 (type III) and 23 (normal lipid values) had slight liver impairment. Patients 16 (type V) and 25 (type IV) had normal liver tests, although one two tests, respectively were missing.

patients were anemic, none because of ling. Hb values were positively and significantly correlated to the creatinine clearance ($r = 0.68$ $p < 0.001$) There was no correlation between Hb concentration and serum lipid values, and no difference in Hb concentration between the three groups of patients. There were no more cases of anemia among patients with hyperlipoproteinemia than among those with a normal serum lipid status.

Although serum concentrations of uric acid, calcium and phosphate were correlated to renal function none of these variables showed intergroup differences or correlation to the lipid status.

DISCUSSION

Whereas hyperlipoproteinemia is of frequent occurence in patients with nephrotic syndrome, only few reports of hyperlipoproteinemia in cases of non-nephrotic renal disease have appeared. Bagdade et al. (1) and Irsigler and Mengele (11) found higher average serum triglyceride levels in their patients with decreased renal function than in control subjects. In the latter study neither the possible inclusion of patients with nephrotic syndrome nor the patients diets are commented upon. Thus, in patients with chronic renal disease hyperlipoproteinemia appears to be of frequent occurrence the incidence of type IV being even higher than in relatives of patients with primary hyperlipoproteinemia (3)

Hyperlipoproteinemia occurs in a number of diseases (6, 12) Patients with diabetes mellitus or thyroid disease have been excluded from this investigation. Except for three cases of hepatic impairment (nos. 11 12 and 23) none of our patients with hyperlipoproteinemia had significant liver damage and none had a large alcohol consumption. Six patients had bacteriuria at the time of investigation, but none had bacteriemia or sepsis hence, the relationship between heavy infection with gram-negative rods and hypertriglyceridemia reported by Gallin et al. (7) cannot explain our observations. Only one of our patients was overweight, and none was on a special diet.

Even though six of our patients had severe proteinuria, the number of patients with hyperlipoproteinemia without proteinuria excludes proteinuria as an important etiologic factor Possibly therefore hyperlipoproteinemia (in particular of type IV) represents an abnormality of lipid metabolism more closely related to the azotemic condition. *On the other hand hyperlipoproteinemia* was not found to be correlated to the degree of renal functional impairment.

In type IV hyperlipoproteinemia (6, 12) as well as in uremia (14 16) glucose metabolism is frequently disturbed. In both conditions diabetic glucose tolerance and hyperinsulinemia may be observed. A raised plasma insulin level will increase hepatic lipoprotein synthesis and, possibly induce hypertriglyceridemia. Similarly an impaired peripheral insulin effect on the target cells might impede the peripheral decomposition of lipoproteins with consequent hypertriglyceridemia. However the relationship between hyperinsulinism, decreased glucose tolerance and hypertriglyceridemia needs further study In our patients glucose tolerance was decreased, but the K-values of normolipemic patients did not differ from those of patients with hyperlipoproteinemia.

In patients undergoing dialysis both Bagdade et al. (1) and Boyer and Scheig (2) found a depressed lipolytic response to heparin (PHLA). Boyer and Scheig do not consider the decreased PHLA to be instrumental in the development of hypertriglyceridemia in such patients. In the primary type IV hyperlipoproteinemia the lipolytic response to heparin is normal. In primary type I and often in primary type V hyperlipoproteinemia an impaired response is found, but characteristically hypertriglyceridemia in these conditions is largely or exclusively due to hyperchylomicronemia.

A diet rich in carbohydrates (as often used in conservative treatment of terminal uremia) might presumably enhance hypertriglyceridemia. The life expectancy of patients with chronic renal disease will be increased by active dialysis. However dialysis does not seem to reduce hyperlipoproteinemia, which has been found as frequently in patients under chronic intermittent hemodialysis (2, 15) as in our patients.

Hence, a number of such patients will have or will develop hyperlipoproteinemia and the associated risk of subsequent arteriosclerotic disease, especially premature coronary involvement. Consequently therapeutic measures aimed at eliminating hyperlipoproteinemia may be considered.

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A RAPID PROCEDURE FOR LABORATORY CONTROL OF SIMULTANEOUS TREATMENT WITH INTRAVENOUS HEPARIN AND ORAL ANTICOAGULANT

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Abstract. A procedure is described by which the laboratory control of simultaneous i.v. heparin and oral anticoagulant treatment may take place without interruption of the heparin treatment. Heparin is administered as an i.v. infusion in a dose which gives a capillary micro thrombin time of 6–13 sec according to the method described in this paper. Under these circumstances the standard Thrombotest (TT) procedure may be used without neutralization of the heparin with protamine. The recommended range of capillary micro thrombin time gives an optimal heparin effect without risk of overdosage. The capillary micro thrombin time may be recorded at the bedside simultaneously with the TT.

In anticoagulant treatment i.v. heparin is commonly used before oral anticoagulation becomes effective. I.v. injections of standard doses of heparin at intervals of 6 hours are often employed. Such uncontrolled heparinization results in considerable fluctuations of the heparin concentration of the blood. Although there is general agreement upon the usefulness of heparinization in thromboembolic states, the proper laboratory control of heparin effect has still not been agreed upon (2, 8). When oral anticoagulants are administered simultaneously with heparin, difficulties arise because heparinization interferes with many of the tests which are used for the control of the oral anticoagulation. Thus, for instance, a pause of 8 hours after the last injection of heparin is recommended before the commonly used Thrombotest (TT) method becomes reliable.

It would thus be important to be able to administer heparin in effective dosage without fluctuations and simultaneously to follow the effect of the oral anticoagulant without interference from the heparin treatment.

In this paper results are reported from the use of a capillary blood micro thrombin time determination method and the TT in the laboratory supervision of combined anticoagulant treatment.

MATERIAL AND METHODS

Reagents

Calcium chloride solution in water, 3.2 and 25 mM.

Heparin solution, 5 000 U/ml (Medica, Finland).

Sodium citrate solution, 3.1%, one part to nine parts blood.

Thrombin solution Topostasin® (Roche, Switzerland), 3 000 U/ml. A stock solution is prepared by dissolving the content of the original vial in 7.5 ml of equal parts veronal acetate buffer and glycerol. The stock solution is stored at −20°C and can be used for 6 weeks (9). The thrombin solution used for analysis is a 1:12 dilution of the stock solution in veronal acetate buffer. The solution is made up in a plastic tube and stored in ice water.

Veronal acetate buffer, pH 7.35 according to Michaelis.

TT reagent according to Owren (Nyegaard, Norway; licence: Medica, Finland).

Phenprocoumon (Marcoumar®, Roche, Switzerland) was used as oral anticoagulant.

Methods

Thrombin time was determined from citrated capillary and venous whole blood by a modification of the method of Seller et al. (9). Venous and capillary samples were taken at the same time. 0.1 ml of capillary and venous blood, respectively, was incubated for 30 sec at 37°C. Then 0.05 ml of thrombin solution was added and the time for coagulation was recorded.

TT determinations were done as recommended by the manufacturer.

Whole blood recalcification time was determined by incubating 0.. ml of citrated whole blood for 60 sec at 37°C, adding 0.2 ml of 25 mM calcium chloride solution and recording the time for coagulation.

Whole blood clotting time was determined by collecting ml of whole blood in a clean test tube and recording the time for clotting in a water bath at 37°C (Lee and White).

Heparinization was done by constant infusion to

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Table I *Effect of heparin added in vitro on thrombin time recalcification time and TT*

Heparin (U/ml blood)	0	0.1	0 2	0.4	0.5	0.6	0.7	1.0
Thrombin time (sec±S.D.)	4 9±0.6	5 7±0.4	6.3±0.5	7.6±1.0	8 4±0.6	9 9±1 7	11 6±2.4	14 7±1.8
Recalcification time, range (sec)	103–105	120–150	143–195	210–270	250–360	270–360	270–360[a]	450–630[b]
TT (%)								
Pat no. 1	8	8	8	8	7.5	7.5	7.5	6
2	12	11.5	11.5	10.5	10.5	10.5	10	8
3	7	7	7	7	6.5	6	5.5	5
4	10	10	10	10	8.5	8.5	8	7
5	91	91	82	91	82	82	77	70

Pat. 2 No definite end-point because of defibrination in test with 1.0 U/ml.
[b] Pat. 4 No definite end-point because of defibrination in test with 0.7 and 1.0 U/ml.

ensure constant concentration of heparin in the blood of the patients. A loading dose of 5 000 U was given as single injection, whereafter an infusion of 30 000 U/24 h was administered. When required, the daily dose was adjusted according to the laboratory results.

Neutralization of heparin was done by addition f protamine hydrochloride (Vitrum) to the TT solvent to give final concentration of 1 U/ml blood in the test.

Siliconized pipettes were used.

Patients

In five subjects the effect of different doses of heparin, added in vitro, upon thrombin time, recalcification time and TT was studied by duplicate determinations. These subjects were either normal volunteers or patients anticoagulated with phenprocoumon. I six other patients effect of i. heparin infusions upon the whole clotting time, recalcification time and thrombin was studied. Thrombin time was always recorded h from capillary and venous blood, taken simultane only The indication for anticoagulation as deep ein thrombosis or myocardial infarction. Altogether 11 test series were done from these patients on different days.

RESULTS

1 *Effect of heparin added in vitro on thrombin time recalcification time and TT*

Blood collected in citrate was mixed with heparin to various heparin concentrations. The values obtained with no heparin and with the different concentrations of heparin were compared. The results are shown in Table I.

The TT values with no added heparin vary according to the status of phenprocoumon treatment of the individual subject. It can be seen that therapeutic heparin concentrations, i.e. 0.1–0.7 U/ml of blood, cause only a negligible effect on the TT whereas, of course recalcification and thrombin times are affected. Lowering of the TT value takes place in the 0.7–1 U/ml range. Good correlation is shown between heparin concentration and the thrombin and recalcification time.

Table II *Effect of i.v. heparin on thrombin time of venous and capillary blood, recalcification time and whole blood clotting time*

Sample no.	1	2	3	4	5	6	7	8	9	10	11
Heparin, i. drip (U/24 h)	35 000	32 500	40 000	35 000	25 000	35 000	40 000	37 500	30 000	37 500	40 000
Thrombin time (sec)											
Venous blood	5	5	5	6.5	5	7	6	5	5	6	5
Capillary blood	5	5	5	7	5	7	6	5	5	5	5
Recalcification time (sec)	105	150	120	290	120	280	210	165	105	135	120
Whole blood clotting time (min)	10	10	8	28	7	21	16	16	7	10	8

2. Effect of heparin in vivo on thrombin time from capillary and venous blood whole blood clotting time and recalcification tim

The tests were done from blood from patients treated by combined anticoagulation with heparin and phenprocoumon. The results are shown in Table II. The good correlation between venous and capillary thrombin times is obvious.

3 Effect of heparin in vivo on TT

The patients were the same as under 2. TT determinations were done from capillary and venous blood with neutralization of heparin with protamine hydrochloride. No difference was found in the TT with and without neutralization of heparin.

DISCUSSION

The optimal mode of administration dosage and control in heparin treatment is still not agreed upon. Heparin as a continuous i.v drip in adequate dosage is the most effective way to achieve the desired result, i.e. to prevent extension of the thrombus and secondary embolism (11 12, 13) Adequate laboratory control is necessary as it has been shown that the required dose of heparin may vary widely between different patients and even in the same individual during the course of treatment (3 12)

The most frequently used tests for the control of heparin treatment are the whole blood clotting time, the recalcification time (4) and the partial thromboplastin time (1 10) The main disadvantage of whole blood clotting time determinations is the duration of the test, which is 15–30 min even if performed at 37 C. The activated partial thromboplastin time determination (1) is a rapid one. It, however requires venous blood and the suspension of partial thromboplastin and kaolin used in this test is unstable (7 14)

The thrombin time as described in this paper is a rapid test and is also sufficiently sensitive for detecting heparin effect in the blood (5) Seiler et al. (9) have shown that the thrombin time from capillary blood correlates well with that from plasma, and that it is a simple and convenient method for the control of heparin treatment. Stewart (11) found a good correlation between the thrombin time of plasma and the whole blood clotting time during heparinization.

Our results confirm the findings of Seiler et al. (9) regarding the good correlation between venous and capillary thrombin times. Our material is too small to allow conclusions regarding the correlation between thrombin time and whole blood clotting time or recalcification time.

The effective dosage of heparin is calculated from clinical results, since experimental conditions resembling those in vivo have not yet been designed. Wessler et al. (15) have shown that an in vitro concentration of heparin which gives clotting times twice the normal is effective in preventing thrombus formation and growth. With the aid of radioactive fibrinogen Kakkar et al. (6) demonstrated inhibition of thrombus growth in heparin-treated patients. The heparin dosage was adjusted not to give thrombin times above 120 sec, with values of 10 to 15 sec in normals by the method used by them. Their method would readily give thrombin times above 120 sec in the capillary micro thrombin range of 6–13 sec by the method we have used. This range was considered desirable in order to ensure an effective heparin concentration of 0.1–0.7 U/ml.

The upper therapeutic limit of heparin concentration is more diffuse. Heparin is a potent anticoagulant, and bleeding may occur in overdosage. This happens readily when standard dosage schedules are applied without laboratory control. Whole blood clotting times above 60 min have been associated with haemorrhage (12). In view of the recalcification and clotting times in our study such a prolongation of the whole blood clotting time is very unlikely if the capillary micro thrombin time is kept within the range recommended by us.

To conclude, it has been shown that the capillary micro thrombin time may be used to indicate adequate heparinization It must be stated that we have measured the effect of heparin, not its concentration. This is, however not essential, as it is the heparin effect which is important in anticoagulation, and not its numerical concentration. If the capillary micro thrombin times are kept within the recommended range of 6–13 sec, overdosage of heparin does not take place, and adequate heparinization is maintained and can be ensured until the TT value reaches 12% or less. By this procedure the basic TT method without neutralization of heparin with protamine is adequate for the control of simultaneously adminis-

tered oral anticoagulant. The capillary micro thrombin time may easily be determined at the bedside simultaneously with the TT

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CLINICAL AND MORPHOLOGICAL SIDE EFFECTS OF BUSULFAN (MYLERAN) TREATMENT

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Abstract. Development of side-effects after long-term treatment with busulfan is described in a 61-year-old woman with chronic myelogenous leukemia. She developed severe pulmonary fibrosis and bilateral cataracts. Cellular atypia in bronchial and uterine cervical epithelium was present after 4 years of busulfan treatment, and in addition cellular abnormalities in the convoluted and collecting tubules of the kidney as well as in the liver were demonstrated. An Addisonian-like syndrome appeared with hyperpigmentation of the skin, fatigue, loss of weight and diarrhoea. Her respiratory symptoms as well as her general clinical condition did not improve after therapy was discontinued. The side-effects of busulfan treatment are discussed and the literature is reviewed.

During the last years we have in at least four patients observed more or less pronounced, but anyhow important, side-effects of busulfan. Cellular abnormalities, mutations, and neoplasms may be induced by radiation, but also by cytotoxic drugs. As far as these drugs are used only for treatment of patients with incurable malignant diseases, there is generally little concern about the possible induction of cellular abnormalities secondary to treatment. As there is today an increase in the use of cytostatically active components not only in malignant but also in allergic, immunologic and other more or less curable diseases, it may be of interest to present a short review of the literature and a report of a selected patient who after long-term treatment with busulfan developed pulmonary fibrosis, epithelial atypia and a wasting syndrome resembling adrenal cortical insufficiency and liver damage.

Busulfan (1,4-dimethanesulfonyloxybutane) introduced in 1953 as an alkylating agent, prevents cell division by combining with phosphate groups of DNA to give denaturation. Since it predominantly inhibits the maturation of the granulocytic cells and often brings about long-lasting remissions in chronic myelogenous leukemia, it has become a popular drug for this disease (22).

Toxic side-effects of busulfan, such as aplastic anemia and thrombocytopenia, have been described but are rare when not more than 6 mg/day are administered. Other well-known side effects are amenorrhea, atrophy of the testes, hyperpigmentation of the skin and cataracts (22).

During the last 10 years several authors have reported 3 additional well-defined complications after long-term treatment with busulfan. These are, first, epithelial dysplasia in many different organs (5, 6, 8, 15, 19, 20), secondly interstitial pulmonary fibrosis (busulfan lung) (2, 3, 8, 9, 13, 14, 17, 18, 20) and thirdly a wasting syndrome characterized by weakness, loss of weight, anorexia, hypotension and hyperpigmentation of the skin. The clinical picture resembles adrenal cortical insufficiency but endocrinological abnormalities have not been found (4, 7, 12, 20).

This report describes a patient with chronic myelogenous leukemia who developed cellular atypia, busulfan lung and a wasting Addisonian-like syndrome after treatment with busulfan for almost 5 years.

CASE REPORT

Clinical course

A 61-year-old woman was admitted to hospital in June 1964 because of weakness, loss of weight, profuse night sweating and recurrent throat infections. Her brother died from a Brill-Symmers lymphoma. She had been cholecystectomized in 1960.

Physical examination revealed a middle-aged woman in good condition. The spleen was moderately enlarged. Lung and heart auscultation normal. BP 135/85 mmHg, Hb 10.5 g/100 ml, WBC 179 000/mm³, mainly immature granulocytes, platelets 93 000/mm³. The diagnosis of chronic myelogenous leukemia was confirmed by bone



Fig. 1. Time relation for busulfan therapy, WBC, Hb and thrombocyte count.

marrow biopsy. Liver function test ere normal. X-ray of lungs and heart as normal.

Therapy with busulfan was given for 5 weeks, 225 mg total. Her clinical condition improved. The WBC pped to 11 000 mm³. Platelets increased to 460 000 and Hb to 12.3 g 100 ml.

Busulfan treatment was again recommended in April 1965 when WBC was 21 700 mm³. Her condition at this time was good. She as then treated with busulfan, 2 to 6 mg day ith only 2 short intermissions until Feb. 1969 when the drug was withdrawn. The total dose of busulfan amounted to 2 800 mg during 4 years and 8 months (Fig. 1).

In June 1967 she developed non-productive cough. Pulmonary X-rays showed streaky infiltrations in the lingular lobe which were not present on previous X-rays. In May 1968 she suddenly developed fever productive cough, vomiting and diarrhea and was admitted to hospital. She had slightly abnormal liver function tests. Pulmonary X-ray revealed pneumonic infiltrations in the left lower lobe. These changes did not show regress after her recovery from the acute illness.

During the autumn of 1968 her condition deteriorated and she complained of non-productive cough, dryness in the mouth and throat, anorexia, loss of weight and fatigue. Bilateral cataracts developed. Pulmonary function studies showed a restrictive ventilatory defect without obstructive components. Atypical epithelial cells in sputum pecimens aroused suspicion of carcinoma.

Since cellular atypia was also present in uterine cervical smears generalized busulfan-induced epithelial dysplasia of the mucous membranes was assumed.

In Feb. 1969 she was again admitted to hospital for further xamination. She was cachectic and dyspneic at rest with hyperpigmented skin and slightly icteric. She had bilateral cataracts. Rales were heard over the lower parts of the lungs. Pulse rate was 120/min and BP 120/75 mmHg.

Laboratory data: Hb 12.5 g/100 ml, platelets 334 000 mm³ WBC 13 800/mm³. Differential count was normal. ERS 35 mm/h, serum creatinine 1.0 mg/100 ml. Serum electrolytes: N 138 mEq/l, K 3.7 mEq/l, Cl 100 mEq l, Ca 4.5 mEq l, P 3.1 mEq/l. Bilirubin 3.2 mg/100 ml. S-GOT S-GPT and alkaline phosphatase activities ere levated. Retention of bromsulfalein after 45 min was 41%.

Pulmonary X-ray demonstrated streaky infiltrations in the lingular lobe and right middle lobe. Pronounced epithelial atypia was still present in uterine cervical smear. No atypia was found in cytologic specimens from the mouth and urinary tract. The Philadelphia chromosome was present in bone marrow cells. Leukocytic alkaline phosphatase activity was normal.

Since the patient was considered to have developed a



Fig. 2. Abnormal epithelial cells with polymorphic and hyperchromatic nuclei in sputum samples.

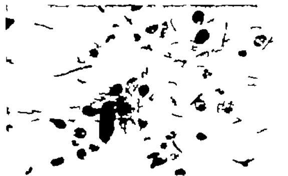


Fig. 3. Cervical smear containing some polymorphic cells with large hyperchromatic nuclei.

busulfan-induced pulmonary fibrosis, the treatment was suspended. During the next month WBC rose from 6 000 to 47 000/mm³. S-GOT, S-GPT and bilirubin returned to normal values, and the retention of bromsulfalein dropped to 14% after 45 min. Alkaline phosphatase activity remained slightly elevated. On leaving hospital her condition had improved somewhat, but she was still dyspneic and easy fatigable.

Because of further deterioration and diarrhea she was again admitted in April 1969. She was then cachectic with ascites and large crural edema. Pulmonary rales were present as before. The pulmonary infiltrations seen on X-ray had not changed since Feb. Hb 10.8/100 ml, WBC 52 400/mm³ with 3% myeloblasts. Hypokalemia but serum electrolytes otherwise normal. In arterial blood pO_2 was 51 mmHg, pCO_2 29 mmHg and pH 7.54. Serum alkaline phosphatase activity was elevated but other liver function tests were normal. Serum γ-globulin was increased and albumin was low.

After treatment with diuretics she initially improved. After two weeks, however, a myeloblastic crisis developed. Treatment with prednisolone and purinethol (mercaptopurine) was started. Two weeks later she suddenly died from gastro-intestinal hemorrhage.

Cytological findings

Advanced epithelial cell changes were present in sputum samples taken in the autumn of 1968 and the spring of 1969. Some extremely large cells with polymorphous and hyperchromatic nuclei were found (Fig. 2). No tumor-like lesions could be detected radiologically in the lungs. Because of this and the information that the patient had been treated for several years with busulfan for chronic leukemia, a cervical smear was requested. This demonstrated advanced abnormalities resembling those found in sputum (Fig. 3). The appearance of pronounced atypia in both sputum and cervix smears made it probable that the changes were busulfan-induced. There were no striking cell lesions in cytological specimens from the mouth and urinary tract. However, only one specimen from each of these sites was examined.

Autopsy findings

Cachectic woman with slight hyperpigmentation of the skin and moderate subcutaneous edema of the legs. She had effusions in the serosal cavities. The lungs had atelectatic areas but no tumor-like lesions in the parenchyma, trachea, or bronchi. No abnormalities were noticed in the cardiovascular system. The spleen was enlarged and the bone marrow hyperplastic. The liver was small (930 g) and firm. No signs of cirrhosis. Macroscopically no lesions were demonstrated in the genital-urinary tract.

Microscopically diffuse leukemic infiltrations were observed in the bone marrow and spleen and to some extent also in the liver and kidney. The histopathological findings confirmed the clinical diagnosis of chronic myelogenous leukemia.

There was moderate diffuse interstitial fibrosis in both lungs. Many alveolar epithelial cells as well as a few epithelial lining cells of bronchioles, bronchi and trachea were markedly atypical (Fig. 4). The affected cells were extremely large with large, polymorphous and hyperchromatic nuclei. The same types of alterations were also found in the epithelial cells of cervix uteri and the proximal and collecting tubules of the kidneys (Figs. 5 and 6). The liver specimens exhibited hepatocellular changes, in principle of the same type (Fig. 7). There was no fibrosis, cirrhosis or fat vacuolization.



Fig. 4. Lung specimen demonstrating a few atypical epithelial lining cells. Note the two large, hyperchromatic nuclei (centre).



Fig. 5. Autopsy specimen from the cervix. The epithelium lining a gland is irregular with large polymorphic epithelial cells.

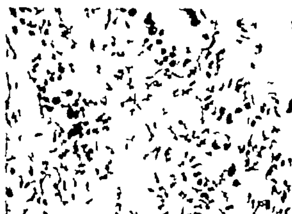


Fig. 6. Advanced epithelial atypia in the collecting tubules of the kidney.

The conclusion of the autopsy findings was: chronic myelogenic leukemia with busulfan-induced pulmonary fibrosis and cellular atypia in the respiratory tract, liver and urogenital tract.

DISCUSSION

In 1961 Oliner et al (17) described two patients with chronic myelogenic leukemia, who developed severe dyspnea fever and dry cough after one year of busulfan treatment. Interstitial pneumonitis with fibrosis was verified by lung biopsy. Busulfan treatment was considered the most likely cause of the pulmonary changes. This assumption has been confirmed by several other authors. The 26 cases reported up to date have been compiled in Table I. In addition Heard and Cooke (8) who investigated lung specimens obtained at necropsy from busulfan-treated patients with chronic myelogenous leukemia, found fibrinous intraalveolar edema, often organized, in 6 of 14 cases. They named the condition busulfan lung.

The clinical picture appears uniform. After busulfan treatment for 1 to 10 years persistent dry cough and progressively increasing dyspnea develop. Sometimes the onset of the disease is acute with fever and productive cough. Persistent rales can be auscultated and chest X ray shows infiltrations, often seen as widespread mottling. Dyspnea due to restrictive lung function and so called alveolar-capillary block, is often severe. Cellular atypia may be found in specimens from the bronchial epithelium. The busulfan-induced fibrosis seems irreversible. There is only one case reported in which the signs and symptoms disappeared after discontinuation of the drug and corticosteroid therapy (17).

Our patient had clinical symptoms and signs of busulfan lung. The diagnosis was confirmed by the autopsy findings.

The pathogenesis of the busulfan lung is not known. Hypersensitivity to busulfan (17) or some inflammatory complications (18) may contribute to the lung disease. Both the disease and cytostatic treatment may cause decreased resistance to infections. More likely the lung lesions may be caused by a toxic action of the drug due to its radio-mimetic properties (13).

Beside the pulmonary symptoms the clinical picture of our patient was characterized by cachexia, severe fatigue long-lasting diarrhea resistant to treatment and hyperpigmentation. The symptoms correspond to the wasting syndrome original-

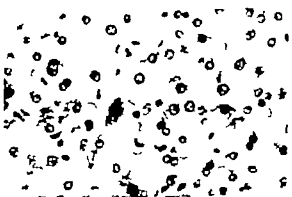


Fig. 7. Autopsy specimen from the liver. Some of the hepatic cells contain large hyperchromatic and polymorphic nuclei.

Table I. *Side-effects in long-term busulfan-treated patients with chronic myelogenic leukemia*

Case no.	Dosage (mg/d.)	Duration of treatment (mo.)	Organs manifesting epithelial atypia	Presence or signs of pulmonary fibrosis	Presence or signs of Addisonian-like wasting syndrome	Reference
1	4–6	10	Lungs, pancreas, kidneys, liver etc.			19
2	2	9	Lungs	Chronic pneumonitis with fibrosis		17
3	2–4	13	Lungs	Chronic pneumonitis with fibrosis		17
4	2–4?	27		Chronic interstitial fibrosis	Weight loss, anorexia, fatigue, hyperpigmentation hypotension	12
5	2–4?	65				12
6	2–4	11				12
7	4	46				12
8	1–6	72	Lungs, pancreas, kidneys, urinary tract, liver spleen, cervix	Atypical pneumonia died in acute respiratory distress		6
9	2–4	30		Chronic interstitial fibrosis	Hyperpigmentation, hypotonia	14
10	4	15		Interstitial fibrosis		14
11	2	48	Widespread epithelial atypias			15
12	6	3			Weight loss, fatigue, anorexia, hyper pigmentation	4
13	6	7				4
14	2–6	46	Lungs, cervix	Persistent non-productive cough	Weight loss, fatigue, anorexia, hyper pigmentation	20
15	4	8	Cervix			8
16	2–8	50	Cervix, urinary tract	X-ray of lungs mottled		8
17	2–6	29	Lungs, cervix, pancreas, adrenals	Lungs mottled on X-ray		8
18	4–8	30	Lungs, cervix, urinary tract	X-ray of lungs mottled		8
19	4–6	16	Lungs, cervix, kidneys, adrenals			8
20	2–8	84	Cervix			8
21	2–4	72	Lungs	Interstitial fibrosis		18
22	2–6	53		Alveolar-capillary block	Weight loss, fatigue, anorexia, hyperpigmentation	18
23	2/3 d.	60		Generalized pulmonary fibrosis	Weight loss, anorexia, hyperpigmentation	7
24	2–4	60	Lungs	Diffuse interstitial pulmonary fibrosis		13
25	2–4	36		Interstitial pulmonary fibrosis		2
26	2–4	120	Lungs	Intraalveolar and interstitial pulmonary fibrosis		3

ly observed by Kyle et al. (12) in 4 patients on long-term busulfan therapy In spite of the Addisonian-like picture, evidence of impaired adrenocortical function has not been obtained (4 7 12) The cause of this syndrome is not known but is believed to be due to some toxic effect of busulfan on cell metabolism (7 12).

In our patient liver damage was present as judged by abnormal liver function tests and ascites. In patients with busulfan-induced wasting syndrome, liver function tests are usually normal except for a rise in alkaline phosphatase activity (7 12). Liver cell atypia as in our case has been described in other busulfan-treated patients (6, 19). With no history of hepatitis, abuse of alcohol or other known hepatotoxic factors busulfan might be considered the most probable cause of liver lesions in our patient.

Treatment with different cytostatics is known to induce cellular atypia (21). In 1960 v Waller (19) described similar epithelial changes caused by busulfan. At autopsy he found cellular dysplasia of the epithelial linings of several organs in a patient with chronic myelogenic leukemia treated with busulfan for 10 months. This has been confirmed by other reports (Table I) which show that busulfan not only acts on granulocytopoiesis but also causes widespread epithelial atypia in many organs and tissues. Thus, cellular changes have been noted in uterine cervix, lungs, urinary tract, intestine, liver spleen, kidneys, adrenal glands, thyroid gland, pancreas, mammary glands and skin (3 4 6, 8 13 15 17 18, 19) In our patient cellular atypia was demonstrated clinically in uterine cervical smears and in bronchial epithelial cells in sputum. At autopsy atypical cells were also seen in the liver and in the tubules of the kidney

The cellular abnormalities do not involve all the epithelial cells. Most of them are normal and some of them only slightly enlarged. The atypical cells are usually very large and often multinucleated, with enlarged irregular and hyperchromatic nuclei. Nuclear vacuolization and inclusions are sometimes observed.

Abnormal epithelial cells found in sputum, in smears of the cervix or in urinary sediments may be very difficult to differentiate from tumor cells. This may lead to an erroneous diagnosis of malignant tumor Therefore it is of great importance that the cytologist is always told whether a patient has been treated with any type of chemotherapeutic agents, especially busulfan.

The possibility that the epithelial atypia may represent early carcinomatous development has been suggested by several authors (1 8, 13 15, 20). It is generally agreed that the microscopic changes are due to the cytotoxic effects of busulfan (5 6 8 19 20) They resemble those which usually are classified as precancerous (6, 8 15 20). Among 26 busulfan-treated patients listed in Table I, carcinoma developed in 3 cases after treatment for 48 60 and 84 months, respectively A connection between busulfan treatment and development of cancer in these patients can neither be proven nor excluded.

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THE RENAL EXCRETION AND TUBULAR REABSORPTION OF CITRIC ACID IN RENAL TUBULAR ACIDOSIS

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Abstract. Three patients with renal tubular acidosis (RTA) have been examined during metabolic acidosis and after correction of the acidosis. Citrate (Ci) clearance was decreased in all patients during acidosis and was not normalized after correction of the acidosis. Renal metabolism of Ci was in the normal range except in one patient in whom the metabolism probably was decreased because of impaired renal function. Tubular reabsorption of Ci was increased independent of the degree of acidosis. We suggest this might be due to decreased tubular synthesis and secretion of Ci.

Urinary citrate (Ci) excretion is decreased in renal tubular acidosis (RTA) (2, 3) and after acetazoleamide blockade of the enzyme carboanhydrase (4, 6). It has not been demonstrated that correction of the metabolic acidosis in RTA normalizes Ci excretion. Previous studies (7) suggested increased reabsorption of Ci in RTA. This finding might indicate reduced tubular synthesis and secretion of Ci. The hypothesis is in conflict with studies in dogs (4) in which no tubular secretion of Ci could be demonstrated.

The present study was undertaken for further elucidation of Ci metabolism in RTA.

MATERIAL AND METHODS

Three patients with RTA were studied during acidosis. The patients were re-examined after partial and complete correction of the metabolic acidosis. The results in one patient (no. 1) have been published previously (2). Renal vein catheterization was performed for determination of the Ci metabolism. Glomerular filtration rate (GFR) was measured as inulin clearance, and renal plasma flow (RPF) was calculated on the basis of para-aminohippuric acid (PAH) clearance and the extraction rate of PAH. The chemical methods used and the formulas for calculation have been described previously (6).

RESULTS

Table I shows the average values for 2–3 consecutive examination periods. All patients were examined at least once after correction of the acidosis. In one patient (no. 1) only GFR and Ci clearance were determined after the correction.

The Ci clearance was significantly reduced in all patients during acidosis (normal Ci clearance = 35 ± 14 ml/min). The Ci clearance increased significantly in one patient (no. 1) after correction of the acidosis, though still in the lower normal range. In the other two patients the Ci clearance was significantly depressed and remained unchanged after correction of the acidosis.

Obviously the Ci clearance was independent of the plasma potassium levels. The Ci clearance was depressed despite normal plasma potassium, and remained unchanged after correction of potassium levels (patient 1).

The amount of Ci metabolized in the renal tubules was within the normal range except in one patient (no. 3) in whom it was significantly decreased and remained unchanged after correction of the acidosis. This patient had an irreversibly depressed renal function, in contrast to the other two in whom GFR increased after correction of the acidosis.

In two patients (nos. 1 and 2) the reabsorption of filtered Ci was significantly increased during acidosis, and remained unchanged after correction of the acidosis. In the third patient with irreversibly reduced renal function the reabsorbed amount of filtered Ci was within the normal range, varying in the three periods between 82% and 69% without any change after correction of the acidosis. The serum Ci levels

diffusion is independent of the presence of other tricarboxylic acids. These processes will, however be influenced by the tubular synthesis of Ci, which is an enzymatic process sensitive to the intracellular pH. In RTA there might be a primary enzyme defect in the tubular cells involving the synthesis of Ci, with increased reabsorption of Ci as a result.

The estimate of the reabsorption of Ci may however be too high if the synthesis and secretion of Ci are reduced in RTA. It has been suggested that Ci is not secreted by the tubules in dogs (4). We are, however not convinced that experimental physiology and clinical research will lead to identical conclusions.

Our results indicate that decreased synthesis and secretion of Ci is the explanation of the reduced excretion of Ci in RTA. The study further indicates that these changes are not merely pH-dependent, since normalization was not obtained after correction of the acidosis. Analysis of Ci in the renal tissue may be necessary for further studies of the metabolic defect in RTA (9).

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IMMUNE PHAGOCYTOSIS IN VIVO OF HUMAN MALIGNANT MELANOMA CELLS

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Abstract. Phagocytosis of tumour cells has been observed in imprint preparations from lymph node metastasis removed from a patient with malignant melanoma. This observation was made when the patient developed a relapse after a spontaneous remission lasting nearly three years. Material from 37 other melanoma patients without a history of tumour remission was studied. Only four showed some degree of tumour phagocytosis. It could be demonstrated by means of the immunofluorescence technique that the freshly biopsied tumour cells were covered with immunoglobulins. After these immunoglobulins had been eluted from the tumour into solution with citrate buffer, pH 3.2, they could react when tested by the indirect immunofluorescence technique with cultured melanoma cells. Furthermore these eluted antibodies were shown to induce phagocytosis in vitro of cultured autologous melanoma cells by heterologous macrophages. The failure of immune elimination of tumour cells and the significance of macrophages in this phenomenon will be discussed in relationship to the observed development of a diminished skin reactivity to dinitrochlorobenzene and a normal response of patient lymphocytes to phytohaemagglutinin in vitro.

In recent years more scientific data has become available showing that not only in the experimental tumour systems in animals, but also in man, immune mechanisms may play an important role in the host's defence system against his own tumour. This applies to melanoblastoma malignum, in which spontaneous regression of the tumour during the course of the disease may reflect the dynamic situation between host and tumour. Furthermore, humoral and cellular immunity against the melanoma cells has been demonstrated by in vitro techniques (1–3).

In this report attention is drawn to a phenomenon of tumour cell phagocytosis which we have observed in lymph node metastases from a patient with malignant melanoma who had previously shown a spontaneous remission. This finding prompted us to study material from 37 other melanoma patients.

The immunological basis of the tumour phagocytosis phenomenon was studied and the results will be described. The possible importance of the phagocytosis phenomenon for the control of the malignant disease that was previously observed in our patient will be discussed.

MATERIAL AND METHODS

Some observations were made immediately after the removal of axillary lymph node metastases from a 46-year-old man with primary malignant melanoma of the right upper leg. In 1965 excision of the tumour with groin dissection was performed. In 1966 he had generally disseminated metastases, but in 1967 a spontaneous remission occurred, leaving a depigmented local area of the skin (Fig. 1 a and b). The patient had a relapse in Sept. 1969 with lymph node and liver metastases. An axillary lymph node biopsy was performed some weeks before he died in April 1970.

Imprint preparations were made from the removed tumour mass and stained with May–Grünwald–Giemsa. Lymph node metastases of 37 other melanoma patients were studied in the same way. These patients did not have a history of spontaneous tumour remission.

A cell suspension was prepared by mechanical disruption of the tumour. The tissue clumps were then removed by passing the cells through a nylon gauze filter as used for blood transfusion purposes. The cells were cultured in M.E.M. Eagle with 20% foetal calf serum. Immunofluorescent staining was performed on the fresh cell suspension. The direct method was used for detection of immunoglobulins on the cells (2). The preparation method of the anti human-immunoglobulin conjugate used in this study has been described in detail elsewhere (5).

Elution of antibodies from the tumour material was



Fig. 1a (1966). Metastases after previously performed excision of a primary malignant melanoma of the right upper leg.

Fig. 1b (1967). A spontaneous regression of the tumour metastases with subsequent depigmentation of the involved skin.

performed at 37°C during 18 hours with citrate buffer 0.02 M, pH 3.2. In control experiments phosphate-buffered saline (PBS) was used instead of citrate buffer. After centrifugation the supernatants were isolated and the pH was corrected to 7.2.

For testing whether the eluates contained specific antibody the indirect immunofluorescent technique was used. Autologous cultured melanoma cells were used as antigen substrate.

In addition the eluates were tested to see whether they could induce immune phagocytosis in vitro. Eluates together with suspensions of cultured autologous melanoma cells were added to monolayer cultures of peritoneal macrophages of guinea pigs. Fresh human AB serum was added as complement source. After 1 1/2–2 hours at 37°C the cover slips were dried and stained.

The patient's ability to develop delayed type sensitivity was tested by measuring skin infiltration after dermal application of dinitrochlorobenzene (DNCB) after prior sensibilization according to the method described by Turk and Waters (6).

The in vitro reactivity of lymphocytes was studied by means of short-term cultures in the presence of phytohaemagglutinin (PHA) using ^{14}C-thymidine uptake by the cells as parameter (4).

RESULTS

The stained imprint preparations revealed numerous large phagocytes with diameters from 30 to 100 μ. The cell nucleus was often pushed against the cell membrane while the cytoplasma was filled with one or more melanoma cells (Fig. 2).

Occasionally the phagocytosed melanoma cells had an intact morphology but more often their structure was altered and eventually only a homogeneous mass of digested tumour cell material remained in the phagocyte. The nucleus of the phagocyte was then flattened as a rim against the cell wall (Fig. 3). In only four of the 37 other patients studied could phagocytosis of melanoma cells be detected though to a lesser extent.

In tissue culture studies no growth of melanoma cells could be obtained initially but after two weeks, during which the culture medium was regularly exchanged, some melanoma cells began to grow out of the cell clumps. Normally it takes at most 48 hours before tumour cell growth occurs.

Application of the immunofluorescent technique showed that many melanoma cells, which could easily be distinguished by their brownish autofluorescence, were covered with immunoglobulins (Fig. 4). The control preparations pretreated with non-fluorescent antibodies did not show membrane fluorescence. However cytoplasmic staining, occasionally seen in some cells, could not be



Fig. 2. May-Grunwald-Giemsa stained imprint preparation from a dissected axillary lymph node. Large phagocyte, cell nucleus pushed against cell membrane, cytoplasm filled with melanoma cells. ×420.

blocked, indicating some non-specific uptake of conjugate by dead cells.

The eluates from the tumour material were tested for the presence of antibodies against tumour membrane antigens. A distinct membrane fluorescence was observed with the citrate buffer supernatants, whereas no antibodies could be detected with PBS supernatants. Phagocytosis of melanoma cells in vitro could be induced with the citrate buffer supernatants of the patient's serum, whereas no phagocytosis occurred with the PBS supernatants.

The DNCB skin test was initially in the remission period, strongly positive. In the later phase of the illness, however when metastases became once again manifest, the skin test became negative. The isolated lymphocytes of the patient responded normally when PHA was added. 8 100 c.p.m. In normal persons the responses varied from 7 300–13 900 c.p.m., while in the controls without PHA the radioactivity varied from 200–1 000 c.p.m.

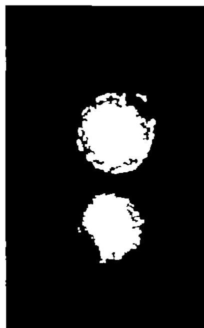


Fig. 4. Detection of immunoglobulins by the direct immunofluorescent technique applied to cell suspensions. The upper melanoma cell shows speckled membrane fluorescence while the other one shows only the brownish coloured autofluorescence. × 210.

DISCUSSION

Imprint preparations made from a metastatic lymph node removed from a patient with malignant melanoma showed numerous pathocytosed tumour cells. As far as we know this phenomenon has not been described before. This observation appeared to be more or less limited to this patient with a history of spontaneous remission of his tumour. Only four of the 37 other melanoma patients studied showed an occasional phagocytosed tumour cell in material from removed metastatic lymph nodes. These patients, however had no history of tumour regression. We assume that tumour phagocytosis may be one of the normal immunological defence mechanisms against malignant cells. It is important to note that phagocytes could easily be seen in imprint preparations but were not so obvious in stained paraffin sections prepared from the same material.



Fig. 3. Phagocytes with nucleus flattened against the cell wall while the cell is filled with homogeneous mass of digested tumour cells. × 210.

The immunological basis of the tumour cell phagocytosis phenomenon resembles the well-known LE cell phenomenon that can be observed in patients with systemic lupus erythematosus. In both diseases the phagocytosis of antigen material is based on autoantibodies. Circulating antibodies active against tumour membrane antigens have been demonstrated in melanoma patients (3). We have shown that immunoglobulins can be bound in vivo by membranes of freshly biopsied melanoma cells. After the cell cultures had been established, immunoglobulins could not be detected anymore on the cell membranes. These findings correspond in this respect to those obtained in Burkitt lymphoma cells by Klein et al. (2).

Our results indicate that the fixed immunoglobulins are indeed antibodies that are bound in vivo to the membrane antigens. This could be demonstrated by eluting the immunoglobulins from the tumour material into solution before testing it by the indirect immunofluorescent technique on autologous cultured melanoma cells. Moreover phagocytosis of the cultured melanoma cells by macrophages could be induced by the eluted antibodies. Thus the same phenomenon observed in the imprint preparations could be reproduced in vitro. It must be stressed that these results could only be obtained when the tumour material was treated with citrate buffer pH 3.2, and not with buffered saline pH 7.2. This indicates that the eluted antibodies must come from antigen-antibody complexes that have been dissociated in an acid environment. Therefore circulating antibodies present in blood vessels or antibodies produced locally by plasma cells in the metastatic lymph node are not likely to be the main source in the above mentioned results. Assuming that the phagocytosis of melanoma cells may have been an essential mechanism leading to tumour regression, the question arises why the tumour escaped the immunological control of the host. In this respect the relationship between the progress of the disease and the capacity of the host to express a cell-mediated immunity is of great interest.

In our patient a conversion of a strongly positive reaction to DNCB to a negative one was observed during the course of the disease. This failure could be due to the lymphocyte as well as the macrophage population of cells. Because the lymphocyte response in vitro to PHA fell within a normal range, a defect or deficiency of the macrophages seems likely. This could account for the inability of our patient to resist his tumour by means of immune phagocytosis and a cell-mediated type of immunity, which could have led to his relapse.

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CARDIAC PACING THROUGH TRANSTHORACIC ELECTRODE IN ACUTE MYOCARDIAL INFARCTION

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Abstract. Among 450 consecutive patients with acute myocardial infarction 20 have been paced by trans-thoracic electrode because of persistent asystole during their stay in CCU. In 19 patients QRS rhythm was induced, but only one survived long enough to have the transthoracic electrode replaced by transvenous one. This patient left the hospital without any disablement.

Asystole may simply be treated by thumping the praecordium. When the blows fail to produce effective myocardial contractions, it is necessary to start external cardiac massage and, as soon as possible electrical stimulation of the heart. In 1952 Zoll (14) introduced stimulation of a heart in arrest by electrodes on the chest. The method still seems to be used in some clinics (2, 15) despite disadvantages such as unreliable cardiac stimulation and unpleasant muscle contractions. To avoid these disadvantages the heart may be stimulated through an electrode transthoracically inserted into the myocardium (1, 7). The voltage may then be kept low. Thevenet et al. presented this method in 1958 (12) and since then successful treatment of patients with asystole in association with both cardiac surgery and acute myocardial infarction (AMI) has been reported (6, 7, 8, 11). Pacing through transthoracically inserted electrodes into the left ventricular cavity has been performed for up to 3 weeks without any complication (9).

The above mentioned results have stimulated the CCU staff of this hospital to use transthoracic pacing routinely in patients with asystole not responsive to thumping of the praecordium. Our experiences after using this method for two years will be presented here.

MATERIAL AND METHODS

Four hundred and fifty patients with AMI were admitted to the CCU f this hospital during the period Jan. 1968 to Dec. 1969 and 20 of these patients ere treated by transthoracic pacing.

If not responding to blows on the praecordium, patients with asystole immediately had transthoracic electrode inserted into the right ventricle. The needle was 175 mm long and had diameter of 1.2 mm. During the passage of the skin and the paracostal tissue it was adapted with trocar hich was retracted before the needle was advanced towards the right ventricle. Through the needle the transthoracic electrode was placed in position. The electrode used in the beginning was of the type described by Lillehei et al. (7), but because of its fragility it was subsequently replaced by an ordinary guitar string, hich functioned well although giving less concentrated current flow. The extra-cardiac electrode (anode), disposable metal needle, was applied subcutaneously below the left costal arch. The electrodes were connected to an external pacemaker generator (EM 138, Elema, Solna, Sweden) with maximal voltage of 18 0. The voltage as well as the stimulation frequency may be varied stepwise. The impulse duration is 1.9 msec.

If pulses became palpable during transthoracic pacing, the patient was transferred as soon as possible to room equipped for fluoroscopy where an electrode was inserted transvenously into the right ventricle.

RESULTS

Of the 20 patients paced through a transthoracic electrode one survived long enough to have a transvenous electrode inserted.

Case report

Female, 68 years old, with mitral stenosis and an infero-apical myocardial infarction one year earlier. She was admitted because of another AMI. Recurrent asystoles during the second day could initially be successfully treated by thumping the chest but had subsequently to be

Table I *Circulatory state preceding the asystole heart weight and estimated size of the infarct at autopsy*
LV = left ventricular VF = ventricular fibrillation

Sex	Age (y)	Heart weight (g)	Infarct size (% of LV myocardium) Recent	Old	Total	Notes
Hypotension or shock						
♂	53	470	40	40	80	
♂	70	630	60	20	80	VF preceded asystole
♂	76	650			65	
♂	80	600	40		40	
♂	90	590	60		60	
♀	60	390	90		90	
♀	61	360	65		65	
♀	69	490	45 + 30		75	VF preceded asystole
♀	70	790	35	35	70	
Pulmonary edema						
♂	63	480	20		20	Rupture of papillary muscle. VF preceded asystole
♂	77	510	15	55	70	Blows effective initially VF preceded asystole
♀	85	440	35		35	Rupture of papillary muscle. Blows effective initially VF preceded asystole
Asystole not preceded by hypotension, shock or pulmonary edema						
♂	46	470	20	30	50	
♂	49	460	35	60	95	
♂	64	425	30	25	55	VF preceded asystole
♂	75	515	65	35	100	VF preceded asystole
♀	66	440	50	25	75	VF preceded asystole
♀	71	310	45		45	Cardiac rupture with tamponade
♀	72	390	45		45	Cardiac rupture with tamponade

ted by external cardiac massage prior to transthoracic ng. The pacing as effective, and 20 min later an docardial electrode had been inserted transvenously into the right entricle and pacing through this electrode was successful. Subsequently during induction of anaesthesia for tracheotomy and respirator therapy tw runs of ventricular fibrillation occurred. One of these stopped spontaneously while the second episode had to be defibrillated.

After 10 days the pacemaker stimulation could be disconnected but the respirator therapy could not be stopped until 4 eeks later because of pulmonary complications. Another month later the patient left the hospital without any overt cardiac failure or angina pectoris. During the next 18 months, before she died in pulmonary edema, she was hospitalized 6 times for brief periods because of cardiac failure.

In this case the transthoracic pacing obviously saved the patient life and she then lived for 18 months

In the other 19 patients the blows on the praecordium were initially effective in 2. In 18 of these 19 patients the transthoracic pacing induced a QRS rhythm but none survived.

Clinical and autopsy data of these 19 patients are presented in Table I The asystole treated was in most cases a complication of either myocardial failure manifested by pulmonary congestion, shock, hypotension or myocardial rupture. In the latter case the rupture was either of the free ventricular wall with tamponade or of a papillary muscle with mitral incompetence. In 8 of the patients ventricular fibrillation preceded the asystole. Of the 5 patients without hypotension, shock or pulmonary edema 2 had a totally or nearly totally infarcted left ventricle.

DISCUSSION

In asystole, thumping of the praecordium can stimulate the ventricles to contract (5 13). This treatment may temporarily maintain a sufficient circulation and thus obviate the risks associated with external cardiac massage such as rupture of thoracic and abdominal organs. However external cardiac massage is often necessary to

prevent cerebral damage and facilitate cardiac treatment. In treatment of asystole four basic treatments may be necessary: the administration of oxygen sodium bicarbonate sympathomimetic amines (epinephrine or isoproterenol) and electrical stimulation. The electrical stimulation may often establish QRS activity but sometimes the myocardium does not get its pump effect back until the other three treatments have been given.

Several successful cases of transthoracic pacing have been reported (4 6, 7 8 11) In most of them, as in this series, the transthoracic electrode was only used until a functioning transvenous electrode had been inserted. Sometimes a transthoracic electrode may stimulate the heart, where a transvenous one fails (10)

Endocardial electrodes may be inserted transvenously into the right ventricle with guidance from the intracavitary ECG only and this is consequently a bedside technique (3) It is, however more time-consuming than the transthoracic route.

The present series of 20 consecutive patients treated with transthoracic pacing for persistent asystole was analysed, as the results seemed to be discouraging.

The analysis showed only 4 of the 20 patients to be potential survivors, and one of these 4 left the hospital. In 7 of the other 16 patients cardiogenic shock preceded the persistent asystole, and in this situation the ultimate result of any pacing method can scarcely be anything but discouraging.

During the same period as the series of 20 patients treated with transthoracic pacing was collected, another patient with ventricular arrest could be treated only by thumping of the praecordium during the time for insertion of a transvenous electrode and establishing a pacemaker-induced heart rhythm.

Case report

Female, 57 years, admitted with an AMI. T hours later complete heart block occurred, lasting 10 sec, and thereafter ventricular asystole. By blows on the praecordium the circulation became effective enough to maintain consciousness, and this could be maintained during the 30 min necessary for insertion of transvenous electrode. At every attempt to stop the thumping the patient became light-headed and no QRS complexes were seen on the oscilloscope screen.

In asystole blows on the praecordium should be tried first. If they are not effective, the chances of restoring the circulation by transthoracic pacing are small. During preparation for transthoracic pacing it is essential to give external cardiac massage, manual ventilation with oxygen and administer epinephrine or isoproterenol and sodium bicarbonate intravenously.

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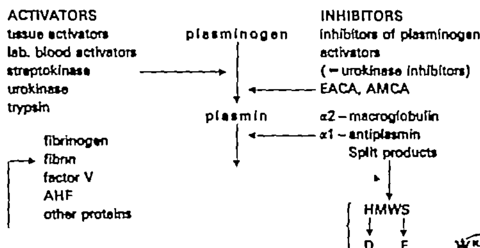
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Urinary tract haemorrhages may be caused by increased fibrinolytic activity
Cyklokapron reduces or arrests fibrinolytic bleeding

In recent years fibrinolytic inhibitors have found widespread use in a number of haemorrhagic conditions, particularly in urinary tract haemorrhages and in connection with prostate surgery. Urine contains urokinase. This enzyme activates the conversion of the plasminogen present in the blood and blood clots into the proteolytic enzyme plasmin, which dissolves clots and thus sustains various types of haemorrhage in the urinary tract. Cyklokapron produces a haemostatic effect by counteracting the activity of urokinase.

The Swedish investigators, Lennart Andersson and Inga Marie Nilsson, have obtained good clinical results by administering Cyklokapron to patients suffering from haemorrhages in the upper and lower urinary tract as well as postoperative bleeding following prostate surgery. Patients suffering from haematuria as a result of general fibrinolysis were also included in the investigation. Bleeding ceased completely in all the patients in the latter group, as was the case with most of the other patients.

the fibrinolytic system



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THE CONCENTRATION OF 21 SERUM PROTEINS IN NORMAL CHILDREN AND ADULTS

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Abstract. By means of Laurell quantitative immunoelectrophoresis albumin, IgG, IgA and IgM have been examined in 258 sera from normal children and adults, while further 17 serum proteins have been determined in 208 of these sera. In comparison with the adults, the children showed lower mean concentrations of prealbumin, α_1-lipoprotein, α_1-antitrypsin, easily precipitable glycoprotein, α_1-antichymotrypsin, Gc protein, haptoglobin, haemopexin, β-lipoprotein, IgG, IgA and IgM, and higher concentrations of ceruloplasmin, α_2-macroglobulin, pseudocholinesterase, complement C'3 and transferrin. Furthermore several proteins showed higher variations of values in comparison with adults. The indication of normal ranges separately for children and adults is therefore necessary. During shorter or longer periods of life, men showed higher concentration than women in respect of prealbumin, albumin, ceruloplasmin, β-lipoprotein, transferrin and IgA and, vice versa, higher concentration was found in women for α_1-lipoprotein, α_1-antitrypsin, ceruloplasmin, α_2-macroglobulin, pseudocholinesterase, haemopexin and IgM. In these periods of life normal ranges for the mentioned proteins should therefore be used for either sex. The variation of the proteins according to sex throughout life is evaluated on the basis of the present knowledge of their function in the organism.

For several years great efforts have been made to determine increasing numbers of proteins in human serum by the immunologic methods (5, 9, 24, 40, 42), whereas the clinician's problem is actually to pick out the proteins which can be useful in diagnosis and evaluation of disease. The function and metabolism of many proteins in the human organism are unknown, hence the determination of the variation of the proteins according to sex and age of normal people and in various diseases can give valuable information. By simultaneous determination of a larger number of proteins in the same serum it is possible to show whether several of these proteins undergo similar changes in case of disease, and whether determination of one of these proteins will be sufficient to follow the pathological process (e.g. orosomucoid during infection) (44).

The purpose of this study has been to determine the 95% range for the concentrations of 21 serum proteins in normal children and adults by means of Laurell immunoelectrophoreses.

METHODS

These have previously been described (40, 41, 42), and therefore only a brief summary will be given here.

Laurell immunoelectrophoreses were carried out on commercial equipment (Dansk Laboratorieudstyr, Copenhagen).

Serum albumin, IgG, IgA and IgM were determined by electrophoresis of serum in agarose gel containing rabbit antihuman albumin, IgG, IgM (Dakopatts A/S, Copenhagen) and IgA (Behring, Marburg, West-Germany) (Laurell rocket immunoelectrophoresis (40)). The anodic mobility of the immunoglobulins was increased after carbamylation of serum with potassium cyanate (39). The relative standard deviation on double applications was 2–6% (43).

Seventeen other α- and β-proteins were identified and quantitated by Laurell crossed immunoelectrophoresis according to Clarke and Freeman (9), using antiwhole human serum from rabbits (Dakopatts A/S, Copenhagen) and carbamylated human transferrin as "built-in-reference" (40, 41, 42). The relative standard deviation on single determinations was about 6% (range 1–18%) depending on the protein in question (43).

Reference. A pooled serum from 1 000 healthy blood donors was used as reference. The concentration of the individual proteins of this serum was stated to be 100 arbitrary units (a.u.), and the concentration of proteins in the unknown sera was expressed in relation to this as a.u. With reference to "Standard serum" (Behring) 16 of the 21 proteins were stated in g (Table II).

Patient sera and reference were stored at −18°C after addition of 1‰ sodium azide until immunochemical analysis was carried out.

Table I. *Age and sex distribution in 208 normal persons, in whom 21 serum proteins were determined. The distribution in a further 50 normal persons, in whom only albumin IgG IgA and IgM determinations were included, given within parentheses*

	Age groups (y)						
	< 1	1–14	15–29	30–44	45–59	> 60	Total
Males	6	20	32 (10)	20 (3)	11 (3)	12 (9)	101 (25)
Females	10	22	25 (9)	33 (4)	12 (3)	5 (9)	107 (25)

MATERIAL

Sera from 258 healthy persons (aged 3 days–93 years) were investigated. Sex and age distribution appears from Table I.

The examined persons comprise: *58 hospitalized children* (aged 3 days–14 years, average 4.4 years), *55 registered blood donors* (aged 19–63 years, average 39), *117 members of medical staff* (aged 18–64 years, average 36), and *28 healthy old people* not confined to bed, from 'De Gamles By' (a home for old age pensioners in Copenhagen, aged 72–93 years, average 78).

All persons were clinically healthy without anamnestic signs of liver or kidney diseases. None of the persons had had intercurrent diseases during the previous month, and in respect of most of them it is a fact that, subsequent to the drawing of samples, they did not show any signs of disease. None of them was given medicine including contraceptives. The children had been hospitalized for diseases which must be considered of no influence on the serum proteins (hernia, enuresis, social reasons).

RESULTS

The mean values (±2 S.D.) of 21 serum proteins in children and adults are stated in Table II.

For 12 of the examined proteins the logarithm of the concentrations was used in the calculations, as their distribution did not differ from the normal distribution in contradistinction to the numerical values (χ^2-test).

The variance of the values in children and adults has been examined through variance analysis (F-test), and the results appear from Table II. For most of the proteins the variance was higher in children than in adults, with the exception of prealbumin for which the opposite was the case. There was no significant difference in variance between children and adults as far as the following were concerned: Gc protein, α_2HS-glycoprotein, ceruloplasmin, complement C'3 and IgM.

The mean concentration in children and adults have been compared by Student's *t*-test, and the results appear from Table II. The children showed a significantly lower ($p < 0.01$) mean concentra-

Table II. *Mean values (±2 S.D.) for 21 serum proteins in children and adults*

	Log values used	Children		Adults	
		Mean	±2 S.D.	Mean	±2 S.D.
1 Prealbumin (g/l)	—	0.17	0.08–0.25	0.25	0.12–0.39
2. Orosomucoid (g/l)	Yes	0.77	0.38–1.58	0.78	0.48–1.26
3 α_1-lipoprotein (a.u./l)	Yes	82	36–189	120*	48–297
4. Albumin (g/l)	—	45.2	34.3–56.0	46.3	37.6–54.9
5. α_1-antitrypsin (g/l)	—	1.41	0.39–2.43	1.71	0.98–2.45
6. Early prec. glycoprotein (a.u./e)	Yes	87	55–139	99	61–159
7 α_1-antichymotrypsin (a. /l)	Yes	102	56–188	115	75–176
8. Gc protein (g/l)	—	0.25	0.15–0.35	0.27	0.17–0.38
9 α_2-HS-glycoprotein (g/l)	—	0.59	0.23–0.95	0.60	0.30–0.90
10 Haptoglobin (g/l)	Yes	1.03	0.18–5.90	1.47	0.58–3.73
11 Ceruloplasmin (g/l)	Yes	0.27	0.10–0.70	0.21	0.09–0.51
12. α_2-macroglobulin (g/l)	—	4.07	2.25–5.89	2.94	1.45–4.43
13 Pseudocholinesterase (mg/l)	—	11	4–18	9	5–14
14. α_2-glycoprotein (a.u./l)	—	90	33–157	88	51–125
15 Hemopexin (g/l)	Yes	0.65	0.35–1.25	0.80*	0.53–1.21
16. Complement C'3 (g/l)	—	0.82	0.57–1.07	0.75	0.35–1.15
17 β-lipoprotein (a.u./l)	Yes	83	35–196	157	90–275
18. Transferrin (g/l)	Yes	2.43	1.46–4.05	2.26	1.52–3.36
19 IgG (g/l)	Yes	7.0	2.8–17.7	10.4	6.8–16.0
20. IgA (g/l)	Yes	0.32	0.02–4.48	1.37	0.54–3.50
21 IgM (g/l)	Yes	0.36	0.11–1.19	0.55	0.21–1.44

p-values: <0.05, <0.01, <0.001

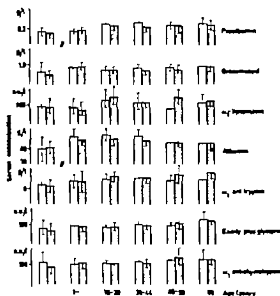


Fig. 1

tion of prealbumin (68% of the concentration in adults) α_1-lipoprotein (68%) α_1-antitrypsin (82%) easily precipitable glycoprotein (88%) α_1-antichymotrypsin (89%) Gc protein (93%) haptoglobin (70%) hemopexin (81%), β-lipoprotein (53%) IgG (67%) IgA (23%) and IgM (65%).

A higher mean concentration in children was found ($p < 0.01$) for ceruloplasmin (128% of the concentration in the adults) α_2-macroglobulin

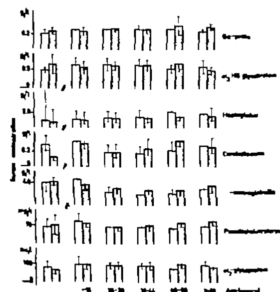


Fig. 2

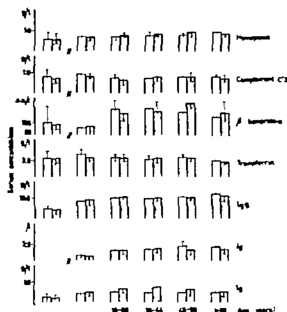


Fig. 3

Figs. 1-3. Mean concentrations for 21 serum proteins (±S.D.) in normal men, □ and women, ▨, of different age groups throughout the life. The number of examined persons appears from Table I.

(138%) and pseudocholinesterase (122%) while the increase of complement C'3 (109% of the concentration in adults) was only significant on the 5% level.

The variation in mean concentration and S.D. for 21 serum proteins with sex and age appears from Figs. 1, 2, and 3.

The higher concentration of β-lipoprotein in women over 45 years (118% of the concentration in men) which appears from Fig. 3, was not significant due to large S.D. and a fairly small number of observed cases.

DISCUSSION

One of the purposes of this study has been to illustrate the variation in serum concentrations which normally takes place for several serum proteins throughout the life of either sex (Figs. 1–3). It will appear that a normal range should be stated separately for children and adults (Table II) and for some proteins for each sex (Table III).

The serum concentration of the proteins throughout the life of either sex can supply information of value in the appreciation of the biological function of the proteins.

Table III *Mean values (± S.D.) for serum proteins which show a difference according to sex at certain periods of life (Student's t-test)*

	Age (y)	Males Mean	Males ±2 S.D.	Females Mean	Females ±2 S.D.
Prealbumin (g/l)	15–44	0.27	0.13–0.41	0.24	0.12–0.36
α-lipoprotein (a.u./l)	45–59	79	35–178	138	76–252
Albumin (g/l)	1–44	48.3	39.9–56.7	46.2	37.8–54.6
-antitrypsin (g/l)	>45	1.51	0.87 2.15	1.96	1.04–2.88
Ceruloplasmin (g/l)	<15	0.32	0.15–0.48	0.23	0.08–0.63
Ceruloplasmin (g/l)	30–59	0.19	0.08–0.42	0.24	0.1 –0.39
α_2-macroglobulin (g/l)	>15	2.62	1.30–3.94	3.22	2.68–4.76
Pseudocholinesterase (mg/l)	>30	9	3–15	11	5–15
Hemopexin (g/l)	15–59	0.76	0.5 1.12	0.82	0.57 1.19
β-lipoprotein (a.u./l)	15–44	166	100–274	148	85–260
Transferrin (g/l)	<15	2.61	1.64–4.16	2.28	1.35–3.83
IgA (g/l)	<15	0.47	0.06–3.90	0.22	0.01–4.00
IgA (g/l)	>45	1.72	0.71–4.14	1.32	0.46–3.76
IgM (g/l)	>15	0.43	0.19–1.19	0.64	0.25–1.61

p-values. <0.05 <0.01 <0.001

Already in the *fifth fetal week* a large number of serum proteins have been found in the new liver cells (13). Somewhat later the immunoglobulins appear after the formation of lymphoid tissue (6, 13) first IgM (10th week) and later IgG (12th week) while IgA does not appear until pretty near the time of birth. For most of the proteins the concentrations in the serum of the fetus increase steadily with increasing age of station indicating a gradual maturation of the thesis of protein (6, 13 14). This process of turation does not end with the birth but continues. Besides the fetus own production of proteins, transport of IgG (15 17) albumin (15) and probably also α_2-HS-glycoprotein (10, 23) takes place from mother to fetus, whereas the placenta seems impermeable to most other serum proteins (31).

At *the time of birth* the concentration of blood in the umbilical cord is for several proteins lower than in the mother's serum (16 17 25) whereas the concentration of albumin IgG α_2-HS-glycoprotein and α_2-macroglobulin are often higher in the child (6, 10 12, 17 21 25 32). By means of a sensitive test (>5 mg IgA/l) IgA, which is often stated to be lacking at the time of birth (2, 4 8 21 27 30 32 38) is detectable in one third of all new-born children (7 35) whereas IgM appears in concentrations corresponding to one tenth of the values in adults (6, 8 21 32, 34 35 38). Haptoglobin is often lacking at the time of birth (25 29) but appears in the course of a few days and quickly increases to measurable values (17 23). The reason may be a considerable release of hemoglobin around the time of birth, and resulting quick elimination of the hemoglobin-haptoglobin complex (11 29). The condition of the immunoglobulins in new-born babies has been particularly well examined. IgG in fetal serum increases with increasing age of gestation (6 18, 37) so that premature new-borns have lower (18 30) and postmature *higher IgG concentrations* than term infants (?). IgM does not change in concentration from approximately the 30th week (6). IgA is higher in postmature children (2) and a relative increased concentration of IgA in new-born babies, simultaneously with normal IgM, therefore adds to the suspicion of postmaturity (2). High concentrations of IgM and possibly also IgA in new-born babies are observed after intrauterine infections (4 27 30 34). Consequently the determination of these immunoglobulins may be a help in deciding whether a prenatal infection in the mother has extended to the fetus, with a risk of permanent damage to the child (4 27 34).

The absence of transport of IgM from mother to fetus means, among other things that the new born child lacks antibacterial antibodies of IgM type against gram-negative rods, which results in special susceptibility to coli infections during the time following birth (15 19). The IgA transmitted

to the child through the IgA-containing mother's milk may be of importance for the protection of the mucous membranes in the gastro-intestinal tract during the time following birth, when the local production of IgA only proceeds gradually.

During the months following birth the maturation of the protein synthesis continues and the serum concentration of most of the proteins increases (8, 12, 16, 17, 21, 23, 32, 35, 38). In premature children this maturation takes longer to set in, and consequently the serum concentrations and the intravascular masses of many serum proteins are lower than in mature children (23). Serum IgG decreases during the months after birth in mature and premature children, which reflects the low IgG in the child and the ceased IgG transport from mother to child (8, 17, 18, 21, 27, 32, 35, 38). A similar fall, but often insignificant, occurs for serum albumin (17).

During the rest of the first 12 months the increase in concentration of most of the proteins continues (8, 12, 17, 21, 23, 27, 32, 35, 38). The synthesis of IgG and IgM is particularly vivid, whereas the IgA synthesis only starts slowly (8, 21, 23, 38). A stimulant for the immunoglobulin synthesis is the constant antigenic influences from, among other sources, the gastro-intestinal and respiratory tracts, hence the large variations in the concentrations of serum immunoglobulin during the first year of life (23). The acute phase proteins reach the same values as in adults during the first year (Figs. 1–3) and likewise high values of haptoglobin (up to 4.5 g/l) can be seen in otherwise normal children during the first year of life (1, 29). This implies a well developed anti-inflammatory preparedness early in life, when so many new influences from outside may release an inflammatory reaction in the child.

After the age of 12 months the serum concentrations for several proteins continue to increase towards the level of adults (Figs. 1–3). For albumin and IgM this level is reached about the age of 1–5 years, for IgG between 5 and 10 years, and for IgA about the age of puberty (21, 23, 32, 35, 38). However, there are exceptions. Already at the time of birth α_2-macroglobulin, which among other things binds the hormone of growth (3, 12), shows higher values in the child's serum than in the mother's; subsequently the concentration continues to increase and reaches a maximum around the age of 1–3 years, whereafter it remains higher throughout childhood than later in life (1), and not until after the puberty are values similar to those of adults reached (12). Also ceruloplasmin, pseudocholinesterase and complement C'3 are higher early in childhood than later in life (Figs. 2 and 3). A higher concentration of IgA has been found in boys and a higher concentration of IgM in girls, which may also be found later in life in men and women (Fig. 3, Table III) (20, 24, 28, 36). This means that the difference cannot be explained by hormonal differences but may be genetically determined.

In adults at the fertile age (20–50 years) it is particularly sexually determined differences between the protein concentrations that are striking (Figs. 1–3, Table III). Several of these differences may be hormonally conditioned, as similar changes are produced by synthetically estrogen-progesteron preparations ('the pill') (increased α_1-lipoprotein, α_1-antitrypsin, ceruloplasmin, α_2-macroglobulin and transferrin, reduced albumin, orosomucoid and haptoglobin (26)) while androgens have produced increased concentrations of prealbumin, orosomucoid and haptoglobin (33). Simultaneously with the increasing concentration in serum of the thyroxin-binding prealbumin in men to a maximum value around the age of 40, a reduction of the thyroxin-binding α-globulin occurs (TBG), whereas estrogens produce the opposite change (33). The changes in prealbumin should be seen in relation to the changes in TBG and not be taken as a sign of changes of the free thyroxin and thus the metabolism of the organism (33). The Gc protein, which is in many respects like the acute phase proteins (44), does not change after the age of 12 months according to sex and age (10). The hem-binding protein hemopexin steadily increases in concentration throughout life and is higher in women (Fig. 3, Table III). Transferrin is often reported to be higher in fertile women than in men (24), which is not confirmed in the present study (Fig. 3). Women aged between 30 and 45 show, however, a large spread in the transferrin concentrations, and it is in this period that the highest values have been observed.

In elderly people there is a slight, but insignificant, increase in some acute phase proteins and IgG (Figs. 1–3). Also α_2-macroglobulin increases after the age of 60 (Fig. 2)—this is reported by other authors as well (12)—but the reason is not known. Serum albumin and transferrin decrease

with age, which implies reduced synthesis in the liver

As stated, the investigation has proved that many proteins change in serum concentration throughout life in each sex, and it would be right to pay due regard to this by stating normal ranges. By means of EDP it is feasible to express the serum concentrations for each sex separately as polynomial functions of age (45) If these equations regarding age are solved, the serum concentration will be corrected for the influence of age. This will be particularly important for the clinically working laboratory in the distinction between normal and pathological values. In the comparison of patients with normal controls, it is of advantage that the factors of age and sex correspond to each other in the two populations.

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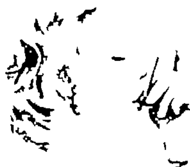
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BODY WEIGHT IN SWEDISH BOYS OF 18

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Abstract. A representative sample of about 3% of practically the whole population of Swedish 18-year-old boys has been examined in connection with enlistment to compulsory military service. The sample consisted of 1 789 boys of 18. The variation of body weight (b.wt.) and other body dimensions such as body height (b.ht.), femoral condylar breadth (right side), chest circumference, waist circumference and muscular strength are presented. The predicted b.wt., reference weight (RW), calculated from b.ht. and femoral condylar breadth are shown in Tables. The quotient between measured weight and RW weight quotient (WQ), is used. The correlation between b.wt. and femoral condylar breadth is higher than that between b.wt. and b.ht. Individual differences, as regards fat and muscular mass of the body are discussed as reasons why in some cases the b.wt. differs considerably from what is predicted from b.ht. and femoral condylar breadth.

In the autumn of 1965 when testing the new enlistment system introduced in Sweden from 1969 a representative sample of boys of 18 was collected in connection with enlistment for military service. Among other variables examined were measurements of certain body dimensions such as body weight (b.wt.) height (b.ht.) skeletal growth and chest circumference. Only few corresponding studies appear to have been published earlier (2, 4 6, 7).

Because of the size and representativeness of the material, and of its homogeneity regarding sex and age, the results may be of some practical interest.

METHODS

Body weight was measured without clothes and reported to the nearest kg. *Body height* was measured without shoes, reported to the nearest cm. *Femoral condylar breadth* was measured with "Caliper" and reported in mm. *Chest circumference* (cm) was recorded at mamillary height after normal expiration, the subjects standing relaxed with the arms hanging. *Waist circumference* (cm) was measured immediately above the crista iliaca under the same conditions. The *maximal isometric muscle strength* (kp) on the right side was recorded in hand grip, elbow flexion and knee extension (8).

MATERIAL

The material, which is described elsewhere (1), consists of 1 789 boys of 18 who constituted a representative sample (3%) randomly selected from the Swedish enlistment population (except the counties of Halland and Gotland) in the autumn of 1965

When presenting relationships between b.wt. and other variables the material has been limited for the same reasons as reported in our study on blood pressure (1). 394 individuals were excluded due to illness or other causes of decreased fitness for service as not being suitable in normal material. Another 80 individuals were excluded since the examination was not complete on all points. Consequently the reported relationships refer to 1 315 individuals.

A comparison has been made between these 1 315 and the excluded 472 individuals. The mean b.wt., femoral condylar breadth and chest circumference were somewhat lower for the 472 excluded individuals than for the remaining group. The differences, 0.53 kg, 0.30 mm and 0.27 cm, respectively are, however not significant (p 0.2). On the other hand b.ht. and waist circumference were significantly lower ($p < 0.05$) for the excluded group. The differences were, however, only 0.77 and 0.61 cm, respectively between the groups. Thus even the significant differences in body dimensions between the two groups are so small as probably to have no practical relevance.

The exact mean age has not been calculated. All the subjects were born in the same year (1947). Thus the variation of age is very small.

RESULTS

The histogram of b.wt. shows a distribution with a slight positive skewness (Fig. 1). The mean



Fig. 1 Frequency distribution of b.wt.

weight is 66.40 kg, S.D 8.61 kg. The number of individuals is 1 787 since information on b.wt. is lacking for two.

The relationships between b.wt. and other variables in the study have been examined. These relationships refer to 1 315 individuals and are highly significant ($p < 0.001$)

The variation of measured b.wt. with other variables is expressed in Table I.

For different combinations of b.ht. and femoral condylar breadth the b.wt. has been calculated according to equation no. 6 in Table I. This predicted b.wt. "reference weight" is shown in Table II. Reference weight is a term first used Behnke et al. (2) Our reference weight (RW) calculated from factors not influenced by obesity (b.ht. and femoral condylar breadth) By dividing the measured b wt of an individual by the RW a quotient is obtained which may be called "weight quotient (WQ) that is

$$\text{weight quotient (WQ)} = \frac{\text{measured weight}}{\text{reference weight (RW)}}$$

The WQ is shown in Table III.

To test the result of the use of WQ this quotient was calculated for 2 818 boys of 18 in connection with enlistment in 1968. The distribution of WQ in this material is shown in Fig. 2. As for b.wt. this distribution is a little skew More than 96% of the material had a WQ within 0.80–1.20

The relationship between b wt. and muscle strength has been examined. The following coefficients of correlation were obtained

b.wt.–hand grip ($r = 0.42$)
b.wt.–knee extension ($r = 0.39$)
b.wt.–elbow flexion ($r = 0.35$)

DISCUSSION

Body weight is dependent both on the dimensions of the body (e.g. height and circumferential measurements) and on relative proportions of different body tissues. Consequently it is not sufficient merely to measure the absolute b.wt. when judging whether the b.wt. is "normal" or not.

Deviations in b.wt. may be a sign of disease or deviating habits (e.g. too high or too low calory intake with the food in relation to the combus-

Table I *Relationships between b wt and certain anthropometric data ($n = 1315$ p 0.001)*

Eq. no.	y	x			R	Eq. of regression line			S
1	B wt.	B ht.		0.51		y 0.665	−51 43		7 02
2	B. wt.	B ht.		0.51		y 0.398	150 92		5 43
3	B. wt.	Fem. cond. breadth		0 62		y 1 146	−44 75		6 44
4	B. wt.	Chest circumference		0 81		y 1.218x	44 16		4 83
5	B wt.	W aist circumference		0 81		y 1 109	16 93		4 81
6	B. wt.	Fem. cond. breadth	B. ht.		0 66	y 0.889	0 340x	80 14	6.18
7	B. wt.	Muscle strength, hand grip		0 42		y 0.331	45 47		7 41
8	B wt.	Muscle strength, hand grip	Fem. cond. breadth		0 66	y = 0 185	0 991	40 46	6.18
9	B. wt.	Skeletal eight		0 62		y 4.223	14 87		6 42

Referred to in the text.
[b] According to v Döbeln s formula (3).

Table II. *Reference weight (RW)*

Femoral condylar breadth (mm)	Height (cm)																	
	140	145	150	155	160	165	170	175	180	185	190	195	200	205	210	15	220	225
75	34.1	35.8	37.5	39.2	40.9	42.6	44.3	46.0	47.7	49.4	51.1	52.8	54.5	56.2	57.9	59.6	61.3	63.0
80	38.6	40.3	42.0	43.7	45.4	47.1	48.8	50.5	52.2	53.9	55.6	57.3	59.0	60.7	62.4	64.1	65.8	67.5
85	43.0	44.7	46.4	48.1	49.8	51.5	53.2	54.9	56.6	58.3	60.0	61.7	63.4	65.1	66.8	68.5	70..	71.9
90	47.5	49.2	50.9	52.6	54.3	56.0	57.7	59.4	61.1	62.8	64.5	66.2	67.9	69.6	71.3	73.0	74.7	76.4
95	51.9	53.6	55.3	57.0	58.7	60.4	62.1	63.8	65.5	67.2	68.9	70.6	72.3	74.0	75.7	77.4	79.1	80.8
100	56.4	58.1	59.8	61.5	63.2	64.9	66.6	68.3	70.0	71.7	73.4	75.1	76.8	78.5	80.2	81.9	83.6	85.3
105	60.8	62.5	64.2	65.9	67.6	69.3	71.0	72.7	74.4	76.1	77.8	79.5	81.2	82.9	84.6	86.3	88.0	89.7
110	65.3	67.0	68.7	70.4	72.1	73.8	75.5	77.2	78.9	80.6	82.3	84.0	85.7	87.4	89.1	90.8	92.5	94.2
115	69.7	71.4	73.1	74.8	76.5	78.2	79.9	81.6	83.3	85.0	86.7	88.4	90.1	91.8	93.5	95.2	96.9	98.6
120	74.2	75.9	77.6	79.3	81.0	82.6	84.3	86.0	87.7	89.4	91.1	92.8	94.5	96.2	97.9	99.6	101.3	103.0
125	78.6	80.3	82.0	83.7	85.4	87.1	88.8	90.5	92.2	93.9	95.6	97.3	99.0	100.7	102.4	104.1	105.8	107.5

tion). The physical activity of the individual also plays a role.

A relationship between obesity and different diseases (with a higher risk of complications and a shorter mean life span) has been reported, e.g. in cardiovascular diseases such as arteriosclerosis and hypertension (5). For evaluation of the prognosis of diseases associated with high b.wt., it is essential to be able to decide to what extent the overweight is of importance.

Fat, muscle and bone tissues constitute quantitatively essential components of the body. The skeletal tissue in the adult individual is relatively constant, while both the quantity of fat tissue (e.g. due to under- or overfeeding) and muscle mass (dependent on physical activity) may vary.

The specific weight of for example fat differs considerably from that of bone. Consequently the relative proportions of different body tissues influence the b.wt. Direct measurements of the fat content of the body are not possible in mass examinations, in which only a short time is available for each individual.

A measure of the development and dimensions of the bone tissue (i.e. the skeleton) is the "sturdiness factor" (6). The sturdiness factor includes the degree of appositional bone growth. In this study the sturdiness factor is expressed as the femoral condylar breadth. Both b.ht. and femoral condylar breadth constitute measures of the skeletal dimensions of the individual. The b.wt. is, however, more closely correlated to femoral

Table III. *Weight quotient (WQ)*

RW (kg)	Measured weight (kg)																		
	35	40	45	50	55	60	65	70	75	80	85	90	95	100	105	110	115	120	125
35	1.00	1.14	1.29	1.43	1.57	1.71	1.86	2.00	2.14	2.29	2.43	2.57	2.71	2.86	3.00	3.14	3.29	3.43	3.57
40	0.88	1.00	1.13	1.25	1.38	1.50	1.63	1.75	1.88	2.00	2.13	2.25	2.38	2.50	2.63	2.75	2.88	3.00	3.13
45	0.78	0.89	1.00	1.11	1.22	1.33	1.44	1.56	1.67	1.78	1.89	2.00	2.11	2.22	2.33	2.44	2.56	2.67	2.78
50	0.70	0.80	0.90	1.00	1.10	1.20	1.30	1.40	1.50	1.60	1.70	1.80	1.90	2.00	2.10	2.20	2.30	2.40	2.50
55	0.64	0.73	0.82	0.91	1.00	1.09	1.18	1.27	1.36	1.45	1.55	1.64	1.73	1.82	1.91	2.00	2.09	2.18	2.27
60	0.58	0.67	0.75	0.83	0.92	1.00	1.08	1.17	1.25	1.33	1.42	1.50	1.58	1.67	1.75	1.83	1.92	2.00	2.08
65	0.54	0.62	0.69	0.77	0.85	0.92	1.00	1.08	1.15	1.23	1.31	1.38	1.46	1.54	1.62	1.69	1.77	1.85	1.92
70	0.50	0.57	0.64	0.71	0.79	0.86	0.93	1.00	1.07	1.14	1.21	1.29	1.36	1.43	1.50	1.57	1.64	1.71	1.79
75	0.47	0.53	0.60	0.67	0.73	0.80	0.87	0.93	1.00	1.07	1.13	1.20	1.27	1.33	1.40	1.47	1.53	1.60	1.67
80	0.44	0.50	0.56	0.63	0.69	0.75	0.81	0.88	0.94	1.00	1.06	1.13	1.19	1.25	1.31	1.38	1.44	1.50	1.56
85	0.41	0.47	0.53	0.59	0.65	0.71	0.76	0.82	0.88	0.94	1.00	1.06	1.12	1.18	1.24	1.29	1.35	1.41	1.47
90	0.39	0.44	0.50	0.56	0.61	0.67	0.72	0.78	0.83	0.89	0.94	1.00	1.06	1.11	1.17	1.22	1.28	1.33	1.39
95	0.37	0.42	0.47	0.53	0.58	0.63	0.68	0.74	0.79	0.84	0.89	0.95	1.00	1.05	1.11	1.16	1.21	1.26	1.32
100	0.35	0.40	0.45	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95	1.00	1.05	1.10	1.15	1.20	1.25
105	0.33	0.38	0.43	0.48	0.52	0.57	0.62	0.67	0.71	0.76	0.81	0.86	0.90	0.95	1.00	1.05	1.10	1.14	1.19
110	0.32	0.36	0.41	0.45	0.50	0.55	0.59	0.64	0.68	0.73	0.77	0.82	0.86	0.91	0.95	1.00	1.05	1.09	1.14

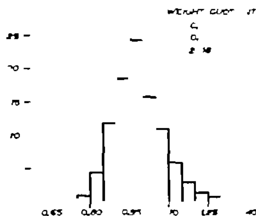


Fig 2 Frequency distribution of WQ

condylar breadth (eq no. 3) than to b.ht. (eq. no. 1) Consequently it seems better to use femoral condylar breadth than b.ht. for prediction of b.wt. in an individual. The time required for measurement of femoral condylar breadth and b.ht. is small. Therefore in practical routine work these dimensions can be used for prediction of ordinary b.wt. The method provides rapid information as to whether the absolute individual b.wt. deviates from the ordinary predicted.

To simplify the use of multiple correlation in eq no. 6 the predicted b.wt. (RW) for different femoral condylar breadths and b.hts. has been calculated in Table II

To make it possible to get a rapid quantitative measure of the individual relationship between measured weight and RW the WQ has been calculated (Table III) In this way individuals with varying height and sturdiness can be compared when *taking into* account *differences in* the skeletal sturdiness factor and b.ht.

The application of WQ to another group of 18-year-old boys (Fig. 2) shows that WQ (using eq no. 6) constitutes a satisfactory measure for classification of b.wt. Consequently WQ is determined and used when evaluating b.wt. at enlistment for compulsory military service in Sweden.

The amount of fat tissue has not been determined. Neither b.ht. nor femoral condylar breadth constitute a measure of the weight of the total body fat. A deviation from 1.00 in WQ may consequently be caused by a deviation in the individual quantity of fat.

The individual muscle mass cannot be measured directly The size and strength of the muscle in the different parts of the body are dependent, among other things, on the degree of physical training of the actual muscles. Measurement of muscle strength in different parts of the body therefore need not be representative of the individual total muscle mass (8) The reported significant relationships between b.wt. and muscle strength (hand grip knee extension and elbow flexion) show that, for example hand grip can be used for prediction of b.wt.

Eq no. 8 shows the prediction of b.wt. from femoral condylar breadth (the sturdiness factor) and hand grip (muscle factor). A comparison with the relationship b.wt. to femoral condylar breadth and b.ht. (eq no. 6) shows the same coefficient of correlation and standard error of estimate i.e. the two relationships are equivalent for prediction of body weight.

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PLATELET PRODUCTION RATE IN POLYCYTHEMIA VERA AFTER MYELOSUPPRESSIVE THERAPY

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Abstract. In nine cases of polycythemia vera (PV) treated with myelosuppressive agents and considered to be in remission the platelet production rate has been determined. Six of them did not have splenomegaly: their values for platelet production rate were within the range of a control group consisting of hematologically normal subjects. In the remaining three subjects with splenomegaly the platelet production rate was above normal. Four of the nine subjects were studied twice, before and after myelosuppressive therapy, and in all of them a considerable fall in platelet production rate was observed after myelosuppression. It is concluded that in PV the platelet production rate after myelosuppressive therapy cannot be evaluated from the peripheral platelet count alone. If splenomegaly persists after remission induced by myelosuppressive drugs, the platelet production rate is likely to be considerably increased, even though the peripheral platelet count has fallen to values within normal limits.

In a previous study (3) it was shown that, in all cases of active polycythemia vera (PV) irrespective of the peripheral platelet count, the platelet production rate far exceeds the range for hematologically normal subjects. The platelet production rate did not change after the hematocrit level had been decreased to normal values by means of repeated phlebotomies (3). Further studies indicated that in active PV there is a tendency for increased platelet production in cases with splenomegaly as compared to those without enlargement of the spleen (4). Moreover, it was observed that the range of individual values for platelet production rate was greater in PV patients with splenomegaly than in those without enlargement of the spleen (4).

Myelosuppressive therapy causes a depression of the bone marrow and shrinkage of the spleen. It was therefore considered of interest to study the platelet production rate in PV patients after myelosuppressive treatment and with special reference to the size of the spleen.

MATERIAL

During the active state of the disease the diagnosis of PV was made according to the criteria previously stated (3). Nine subjects, six males and three females, were studied. Four of the patients had been treated with busulphan, three with chlorambucil and two had received ^{32}P. The patients were considered to be in remission if reduction in the venous hematocrit to $\leq 50\%$ had been induced by myelosuppressive therapy without the aid of phlebotomy. Concomitantly, normal or slightly subnormal peripheral white cell and platelet count was present, and the patients had no symptoms which could be ascribed to PV. In spite of iron administration and in the absence of blood loss there was no rise in their hematocrit values. Table I presents data as to age, sex and therapy. Four of the subjects (nos. 6–9) were studied twice, while in the active stage of the disease and after myelosuppression (Table II).

At the time of the present study all subjects were considered to be in a stationary state. There was no history of blood loss and no clinical signs of infection or thrombosis. None were receiving anticoagulant therapy.

The patients were considered not to have splenomegaly if the spleen was not palpable and if X-ray did not reveal any enlargement of the spleen (3). If this was not the case, the patients were classified as having splenomegaly.

Sixteen hematologically normal men of comparable age were chosen as control subjects. Eight of these were normal volunteers, and the remaining eight suffered from mild to moderate cardiovascular diseases (2).

METHODS

All platelet survival studies were carried out with autologous platelets. Acid ACD (1) was used as anticoagulant and ^{51}Cr as the platelet label. The details of the labelling technique, the procedure for blood sampling and dif-

Table I *Platelet survival studies in nine patients with PV in clinical remission after myelosuppressive therapy*

Subject no.	Age (y.)	Sex	Therapy	Hct ( )	Platelet count (per µl blood)	Splenomegaly	MLS (d.)	Recovery ( )	Platelet production rate (no 10⁹ d.)
1	5	♀	Busulphan	41	144 000	0	7.5	33	19
2	58		Busulphan	41	210 000	0	4.1	72	19
3	63	♂	Busulphan	48	251 000	0	5.3	40	35
4	69	♂	Chlorambucil	39	180 000	0	6 0	32	33
5	66	♂	Busulphan	43	139 000	0	4 8	4*	28
6	72	♀	MP	40	150 000	0	3.8	47	25
7	71	♂	Chlorambucil	50	193 000	+	5 9	13	115
8	49	♂	MP	50	707 000	+	5.9	19	78
9	72	♂	Chlorambucil	37	249 000	+	3 7	20	116
Mean					191 000		5.2	35	52
S.D					4[illegible] 000		1.25	17 9	40
S.E.					14 000		0.42	6 0	13
Controls (= 16)									
Mean					181 000		6.4	53	23
S D					25 000		1.53	7 1	6
S.E.					6 000		0.38	1.8	

ferential centrifugation and the principle for the calculation of platelet recovery has been described previously (2, 3).

For the determination of platelet mean life span (MLS) according to the principle of Mills (5) the following arbitrary mathematical function was used:

$$Y(x) = Y(0)\left(1 - \frac{x}{T} - A(1 - e^{-\alpha x})\right) \quad \begin{cases} 0 < T \\ 0 \le A \\ 0 \le \alpha \end{cases}$$

With a digital computer (IBM 360 65) experimental data were fitted to the above function by the least-squares method. Platelet MLS is given by $Y(0)/Y'(0)$. The platelet production rate (P) and the platelet destruction rate (D) are obtained from $P = D = N/\text{MLS}$, where N denotes the total number of circulating platelets including those in the exchangeable splenic pool (2, 3).

Platelet counting was performed with a Coulter Counter Model F on venous blood collected in EDTA powder as reported previously (2, 3). The platelet counts are the means of triplicate determinations on day zero.

Unless otherwise stated, mean values ± standard error of the mean (S.E.) are reported. The mean values were tested with Student's t-test. The difference of means is considered to be statistically significant if $p < 0.05$.

RESULTS

Platelet count The mean peripheral venous platelet count in the group of PV patients studied after myelosuppressive therapy was 191 000 ± 14 000 with a range of 139 000–[illegible]51 000 µl (Table I) The mean platelet count for the four subjects studied during active disease was 635 000 ± 157 000 µl (Table II)

Platelet recovery The range for platelet recovery in the control group was 42–67% (mean 53 ± 7 1 S.D) The individual recovery values

Table II. *Platelet survival studies in four cases of PV before the institution of myelosuppressive therapy*

Subject no	Hct[a] (%)	Platelet count (per µl blood)	Splenomegaly	MLS (d.)	Recovery ( )	Platelet production rate (no 10⁹ day)
6	[illegible]	334 000		5.9	[illegible]	46
7	43	772 000		3.8	34	37*
8	60,46	4[illegible]2 000		4 7	20	228
9	53/38	1 012 000		3.5	19	601
Mean		635 000		4.5	23	317
S.D		315 000		1.08	6 9	227
S.E.		157 000		0.54	3 4	114

[a] The subjects for whom two hematocrit values are given were studied twice at a high hct level and after phlebotomy at a normal hct level. In these cases the mean values of the duplicate studies are reported.

for the nine PV patients studied in remission are given in Table I. Six of the patients were considered not to have splenomegaly. Three of these subjects (nos. 2, 5 and 6) had recovery values above the lower range of the controls. In the remaining three PV patients without splenomegaly (nos. 1, 3 and 4) a slight to moderate decrease in platelet recovery was observed. The recovery values for the three splenomegalic subjects (nos. 7–9) were substantially reduced.

Platelet mean life span. The mean platelet MLS for the control group was 6.4 ± 0.38 with a range of 3.7–8.7 days. The mean value for the PV patients in remission was 5.2 ± 0.42 with a range of 3.7–7.5 days. All individual values were within the range of the control subjects (Table I). The mean for the PV group was not statistically different from the mean of the controls ($0.10 > p > 0.05$).

Platelet production rate. The range for platelet production rate in the control group was $11 \times 10^{10} - 35 \times 10^{10}$/day (mean $23 \times 10^{10} \pm 6 \times 10^{10}$ S.D.). The individual values for the nine PV patients during remission are given in Table I and Fig. 1. In all six non-splenomegalic PV patients the values for platelet production rate were within the control range. In the remaining three splenomegalic subjects the platelet production rate was still substantially elevated ($78 \times 10^{10} - 116 \times 10^{10}$/day). In all the four cases studied twice, both during the active stage of the disease and after myelosuppressive therapy, a considerable decrease in platelet production rate was observed (Tables I and II).

DISCUSSION

Phlebotomy is a valuable therapy in PV, since symptoms due to plethora and elevated blood viscosity are immediately relieved. After repeated phlebotomies a state of iron deficiency with impaired erythropoiesis develops, and the hematocrit level may remain within normal limits for a long time. Granulopoiesis and thrombopoiesis are usually not affected and there is no appreciable decrease of the proliferating organs. Platelet production rate (*P*) has been shown to remain unchanged after normalization of the hematocrit by repeated phlebotomies (3). Therapy with myelosuppressive agents on the other hand decreases the overall myeloproliferative activity.

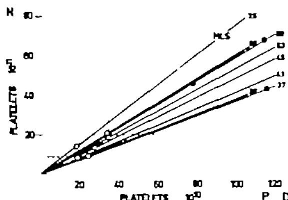


Fig. 1. The relationship between *N*, MLS and *P* (= *D*) in nine cases of PV studied after myelosuppressive therapy. The figures give the platelet MLS for the subjects. ○ no splenomegaly; ● splenomegaly. The areas between the dashed lines denote the ranges for *N* and *P* in 16 hematologically normal control subjects of comparable age.

It has been shown that *P* in all cases of active PV exceeds the range for hematologically normal subjects (3, 4). The present study shows that, after myelosuppressive therapy, *P* was within the control range in all six PV patients who did not have splenomegaly. In the remaining three patients with splenomegaly *P* was still elevated. However, in all four cases studied twice, before and after myelosuppressive therapy (nos. 6–9), a marked decrease in *P* was found.

The calculation of *P* is dependent on *N* and MLS. *N* is obtained from the venous blood platelet count multiplied by the total blood volume and corrected for the platelets in the exchangeable splenic pool. The size of the latter can be indirectly deduced from platelet recovery. All nine subjects studied after myelosuppressive therapy had a normal peripheral platelet count (mean $191\,000 \pm 42\,000/\mu l$ S.D., range $139\,000–251\,000/\mu l$). In the six non-splenomegalic PV patients *N* was within (or almost within) the control range (Fig. 1). This was also the case in those three subjects classified as non-splenomegalic, but in whom platelet recovery was slightly or moderately reduced. This subnormal platelet recovery suggests the presence of an increased splenic platelet pool and probably an increase of the spleen size, even if this increase was not demonstrated by X-ray examination (4). In the three splenomegalic PV patients *N* and the splenic platelet pool were considerably increased (Fig. 1).

In all PV patients studied after myelosuppressive therapy the individual values for platelet MLS were within the control range The mean platelet MLS of the PV patients was lower than the mean of the controls, but the values did not differ significantly The most impressive difference between the non-splenomegalic and splenomegalic subjects was that *N* and *P* were considerably higher in the latter Of the two parameters, *N* and MLS, which influence the platelet production rate platelet MLS was of lesser importance.

The present study shows that the platelet production rate after radio- and chemotherapy cannot be evaluated from the level of the peripheral platelet count alone. In the presence of splenomegaly and consequently an increased splenic platelet pool, *P* is expected to be considerably increased although there is a normal peripheral platelet count. Not until *N* is normal is *P* likely to be within normal limits.

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MEDICAL TREATMENT AND MORTALITY IN CARDIOGENIC SHOCK

Metaraminol Compared with Combined Phentolamine and Norepinephrine Glucagon Therapy

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Abstract. 110 patients with cardiogenic shock, mostly due to myocardial infarction, were treated with pure α- and β-sympathomimetic. The subsequent 137 patients received combination of sympathomimetic and α-sympatholytic. In the series treated with the combination, glucagon was used in cases of heart failure. Comparison of the mortality figures after one week and after one month revealed no difference between the series. Neither the sympatholytic used nor the glucagon given in cases of failure increased the survival rate. According to current investigations the favourable results obtained with sympatholytic and glucagon in small materials are not confirmed by the findings in larger materials like ours of cardiogenic shock with myocardial infarction as the primary etiology.

Since the beginning of the 1950's the severity of cardiogenic shock has been evaluated on the basis of the BP level, and the effect of treatment has been measured by the success in restoring the normal BP. Around that time the "vasopressor" drugs, particularly norepinephrine and metaraminol, were introduced (1, 5, 12, 17, 19, 25, 30, 32, 35, 38, 53). Their use was based on the assumption that a rise in arterial BP increases the effective systemic blood flow and, on the other hand, the vasoconstriction brought about by these drugs facilitates coronary flow because of the rise in coronary perfusion pressure (14). It was further assumed that the work of the heart does not increase out of proportion. Investigations into traumatic and haemorrhagic shock later suggested that the various measures causing vasodilation might also improve the survival rate. During the last ten years intensive work has also been carried out on the use of vasodilators in cardiogenic shock, particularly after open-heart surgery (24, 33, 41). Besides the individual pharmaceutical agents mentioned, combinations of vasodilators and ionotropic drugs have also been used (7) and in recent times, some attention has been paid to the effect of glucagon in cardiogenic shock.

After 1966 mainly two different regimens have been used at our hospital in the treatment of cardiogenic shock. Metaraminol, used earlier, has been later changed to a combination of noradrenaline and phentolamine, to which combination glucagon in cases of heart failure has been added. In the following we report our experiences and compare the efficacy of the regimens mentioned. Despite the seemingly unambiguous criteria, cardiogenic shock is difficult to diagnose (23, 43, 46, 49, 50), etiological factors are numerous, examination is difficult, and reference materials without bias are hard to obtain (37). Recovery or death from cardiogenic shock, however, can be recorded indubitably.

MATERIAL AND METHODS

The University Central Hospital in Oulu is responsible for treating all patients with cardiogenic shock fit to be transported from an area with about 160 000 inhabitants. Patients were divided into two series. During the period 1.11.1966–15.10.1968 (23 1/2 months) there were 110 patients with cardiogenic shock. They will be called series I. During the period 16.10.1968–31.7.1970 (21 months) there were 137 patients with cardiogenic shock. They will be called series II. A subgroup of this later series consists of the 74 patients treated, in addition, with glucagon. They will be called series II Gl.

In both series essentially similar criteria for cardiogenic shock were used: decreased cuff and pulse pressure, cold extremities, and decreased diuresis. The usual criterion was that the systolic BP at the same time was less than 90 mmHg. In known hypertonic, however, the BP may have been 90 mmHg or higher, yet cardiogenic shock

Table I *Age and sex distribution in different series*

	Series I (%)	Series II (%)	Series II GI ( )
Women	22	33	40
Men	78	67	60
Age (y) 50	19	12	11
50–59	27	25	22
60–69	36	40	35
≥70	18	23	32
No of pats.	110	137	74
Mean age (y)	58.6	61.8	63.9

was diagnosed if the other criteria were fulfilled. The patients have been either alert or obtunded. All patients with simple hypotonia, without any other criteria of shock, have been excluded.

The age and sex distribution of the patients and their mean ages are presented in Table I. The two series are comparable but among subjects in series II GI there were more above 70 years of age than in the other groups. Some characteristics are presented in Table II.

The treatment of cardiogenic shock in the different series is presented in Table III. The pressor agent used for series I was metaraminol, and for series II combination of levarterenol and phentolamine. An attempt was made to keep systolic BP around 100 mmHg in both series. The systolic BP of hypertonic patients was raised to even higher values if the shock did not improve at the level of 100 mmHg. Pain, nausea and hypoxia were treated in the same manner in the two series. Patients with congestive heart failure were digitalized. Recently glucagon has been used for the most critical cases in an attempt to obtain inotropic action by ways other than those digitalis. Similarly glucagon has been used to cover period of 20–30 min which elapses before the effect of digitalis is f h (21).

Thorax X-rays and central venous pressure were used when diagnosing heart failure and evaluating the effectiveness of the treatment (57). When the values of central venous pressure were below 1 cm of saline hypovolaemia could be suspected, and when the values rose above 15 cm observations for the possible onset of

Table II *Characteristics of the shock in different series*

	Series I (n)	( )	Series II ( )	( )	Series II G ( )	( )
Pump failure	70	64	92	67	52	70
Electrical failure	40	36	45	33	22	30
Myocardial infarction	90	82	117	85	64	86
Treatment delayed	14	13	26	19	16	22
Resuscitation with primary success	39	36	48	35	36	49
DC shock used	35	32	56	41	37	50
Total no. of pats.	110		137		74	

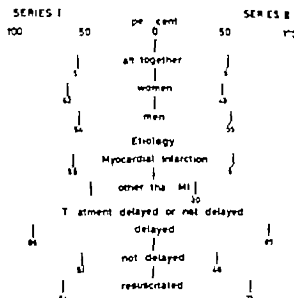


Fig. 1 Mortality in one week in series I and II.

failure were begun. Since a long duration of interstitial pulmonary oedema in the X-rays has been noted even after effective treatment (42), and similarly great variations of the values of central venous pressure during cardiogenic shock (2, 20), the condition of the patient was also evaluated on the basis of clinical criteria.

Arrhythmias, overdosage of digitalis, and thromboembolic complications were treated in the same way in the two series. All the patients with acidosis were given 7 % sodium bicarbonate

RESULTS

Fig. 1 shows the 1-week mortality in series I compared with series II. In series I the 1-week mortality of females was higher than in series II, although the difference is not significant. With an etiology other than myocardial infarction the survival of the patients in series I was poorer than in series II but again the difference is not significant. With myocardial infarction as the etiology the two series did not differ from each other. If the treatment was delayed, i.e. if the patient was already suffering from shock when he entered the hospital, mortality was the same in the two groups.

Fig. 2 shows the comparison of the 1-week mortality figures of the patients in series II who received glucagon and the corresponding figures of those managing without glucagon. Pressor treatment was the same in series II and II GI. There is a considerable difference in 1 week total

Table III. *Therapy of cardiogenic shock in different conditions*

Condition	Series I	Series II	Series II Gl
Shock	Metaraminol	Levarterenol + phentolamine	Levarterenol + phentolamine
Pain	Meperidine	Morphine	Morphine
Nausea	Promethazine	Promethazine	Promethazine
Hypoxia	Oxygen 100% by nasal catheter by tight face mask, with pressure- or volume-controlled ventilator according to P_{O_2} values		
Congestive heart failure	Digitalization	Digitalization	Digitalization and glucagon 3 mg continued with 1 mg at 10 min intervals
Arrhythmia			
Atrial tachycardia	Digitalization	Digitalization, propranolol	Digitalization, propranolol
Atrial flutter	Digitalization	Digitalization, propranolol	Digitalization, propranolol
Atrial fibrillation	Digitalization	Digitalization, propranolol	Digitalization, propranolol
Ventricular tachycardia	Lidocaine	Lidocaine	Lidocaine, propranolol with glucagon
Ventricular fibrill.	DC shock	DC shock	DC shock
Sinus bradycardia	Atropine, pacing	Atropine, pacing	Atropine pacing
A–V block, total	Pacing	Pacing	Pacing
Digitalis toxicity	Potassium chloride, propranolol	Potassium chloride, propranolol	Potassium chloride, propranolol
Thromboembolism	Heparin, phenindione	Heparin, phenindione	Heparin, phenindione
Acidosis	$7\frac{1}{2}$% sodium bicarb.	$7\frac{1}{2}$% sodium bicarb.	$7\frac{1}{2}$% sodium bicarb.

mortality the patients who needed glucagon had mortality figures statistically higher than those not receiving glucagon. A similar difference in mortality can be noted in the male material. If the etiology is myocardial infarction there is a significant difference in mortality but in cases with other etiology 1-week mortality is the same in all the series. Whether treatment is delayed or not, if patients have been resuscitated, mortality figures are equally high in the groups treated with glucagon and in the group not given glucagon.

Fig. 3 shows that there is no significant difference between the total mortality figures after one week and after one month. The two sexes die in the same proportions in either series. The etiology whether myocardial infarction or other causes no difference between the 1-week and 1-month mortality. Nor does delay of treatment bring about any difference in the mortality rate. The same concerns patients resuscitated.

DISCUSSION

Cardiogenic shock is defined as a state in which the heart cannot pump enough to meet the needs. The most common cause of this is myocardial infarction or an operation on the heart. The ischaemic myocardium does not contract properly and may even bulge out during the systole (55). This is followed by activation of the stretch receptors in the bulging myocardium, followed in turn by vasodilation (13) which then prevents the compensatory vasoconstriction from being sufficiently effective. Half of the patients with cardiogenic shock following myocardial infarction are found to have normal systemic vascular resistance, which seems to be an insufficient response to the drop in cardiac output (20, 27).

If the supportive measures normally used in the treatment of cardiogenic shock do not lead to prompt success, the situation is problematic. The question is whether it is better to concentrate primarily on the attempt to maintain the arterial BP or to try to dilate the contracted arteriolar beds in the kidneys, liver bowels, etc thereby reducing tissue anoxia in these vital organs. Stabilization of arterial BP may lead to peripheral vasoconstriction, while on the other hand peripheral vasodilation may lead to a decrease of arterial BP

Myocardial infarction and cardiogenic shock are followed by a strong sympathoadrenal response to hypotonia (16). Catecholic agents bring about spasms in the arterioles and venules, and when acidosis develops, vasodilation takes place in the arterioles but not in the venules. Thus the blood

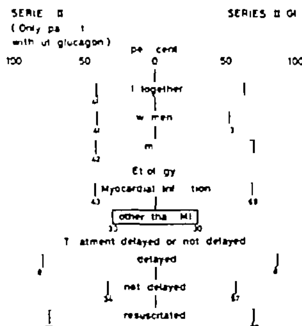


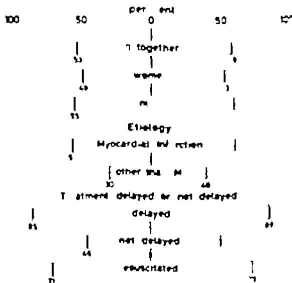
Fig Mortality in one week in series II treated ith and without glucagon.

has easy access to the capillary bed but difficulties in getting out. Hydrostatic pressure increases, and plasma escapes into the interstitial space Such a state has been called stagnant anoxia (34) On these grounds vasod lators and heart-stimulating agents have been recommended when treating cardiogenic shock.

Myocardial infarction is the most common etiology in cardiogenic shock, in addition to the patient often has a serious coronary disease affecting even the vessels which responsib.e for the intact myocardium (6) Thus there is reason to fear that the coronary flow might depend on the pressure (39). The coronary arteries collapse when the mean pressure comes down to 15–50 mmHg, and after this critical level has been reached, coronary flow ceases (9 11) Patients with cardiogenic shock probably ha e atherosclerotic constrictions even in the very peripheral coronary network which makes it possible that their perfusion ceases even at relatively high alues of arterial pressure It therefore seems important that BP should be stabilized at a relatively high level, at least as high a systolic value as 80–90 mmHg Values higher than this however cannot be used, because the after-load and oxygen consumption increase (48). On the basis of the above factors vasopressor agents are used in cardiogenic shock.

The use of vasopressors which was started nearly 20 years ago, first gave the impression that the survival rate no less than doubled (1 15 ... 30 38) Yet it has been maintained that there is no definite evidence showing that these drugs have improved survival (4 35 36). It was noted that it was relati ely easy to increase arterial BP with these pharmaceutical agents, but that the increase of pressure did not, in fact, signify any improvement of circulation as evaluated by clinical criteria and the mortality rate (2, 5) In his latest paper Kuhn (29) reviews 18 series consisting of a total of 268 patients. In this material effective pressor response was obtained in 77 % and the shock was relieved in 50% while mortality varied from 14% to 100% being 60% on the average. Véghelyi (51) collected data on about 000 patients, among whom the survival rate of those treated with vasodilators was of the order of 80% whereas the mortality rate of those treated with vasopressors was 80 %. It seems that the results depend on the material and that different materials are not comparable with each other

During the last few years combinations of a vasopressor and a vasodilator have been introduced in the therapy of cardiogenic shock, according to the receptor theory (3) mostly a combination of a sympathomimetic affecting the α and β-receptors and an α-sympatholytic. The purpose of this is to utilize the positive inotropic



F g 3 Comparison of the mortality rate in one ek and in one month in series II

cardiac effect of the sympathomimetic and the antivasoconstriction effect of the sympatholytic. The combination of drugs has been found to have the expected effect both in experimental work on shock (54) and in clinical experiments (7 8) Phentolamine has often been used because it can be administered i.v and, being a drug with short term action, can be easily controlled. Phentolamine can also be recommended for its β-sympathomimetic effect, which was recently discovered and which would increase the cardiac output. It has further been used for its antiarrhythmic effect (18). Theoretically it would be possible to use this drug combination to control the amount of α-sympatholytic according to need. Yet it has also been reported that this is difficult to achieve in practice (28).

The present material consists of a prospective collection of patients treated with a pure α- and β-sympathomimetic, and another series treated with a combination of a sympathomimetic and an α-sympatholytic. The latter series also includes a subgroup of patients treated with glucagon. A natural implication of the definition of the state and the set of indications for the drug is that the series treated with glucagon contains more patients with pump failure than the other groups. The failure group treated with glucagon initially showed better primary success with resuscitation than the other series. It was further noted that the patients treated with glucagon already had shock when they entered the hospital, and that DC countercurrent shock was most frequently needed in their treatment.

Examination of the 1-week mortality rates of the patients treated with a sympathomimetic alone and those receiving a combination of a sympathomimetic and an α-sympatholytic revealed no significant differences. Mortality was the same irrespective of sex, and also irrespective of whether the etiology was myocardial infarction or not. High mortality was noted consistently in the patients who possibly already had the shock developing before they entered the hospital.

Comparison of the 1-week mortality figures of the patients who required glucagon and those who managed without in the series treated with a combination of a sympathomimetic and an α-sympatholytic revealed significant differences. More patients were lost during one week in the whole series requiring glucagon than in the others. The difference is significant in the male material, but not in the female. A further significant difference was noted in the cases with an etiology of myocardial infarction. deaths were more numerous in the group treated with glucagon. In the cases without myocardial infarction no difference was noted This agrees with the known fact that the dynamics of cardiogenic shock differ in such cases. If coronary arteries are not affected, vigorous peripheral vasoconstriction is fully sufficient to relieve the shock (34) which is not the case in cardiogenic shock brought about by myocardial infarction. If the patient already has primary shock when he enters hospital, glucagon is not found to bring about any success, the survival rate being equally low in both groups.

The recent acute experiments with glucagon in cases of refractory cardiac failure and cardiogenic shock have given promising results BP has been found to rise, excretion of urine to increase and symptoms of failure to decrease Arrhythmias have not been noted, and even antiarrhythmic effects have been observed (44 56) Large-scale clinical experience is still scanty and the effect on mortality in cardiogenic shock is not yet known In the present series glucagon was used systematically to treat failure in cardiogenic shock. The results seem discouraging. Glucagon had no favourable effect on the prognosis of the patients. It has been considered highly doubtful that improved intensive treatment could have any effect on the survival in cardiogenic shock (5 36). The possible bias in the present work, as well as in other works on shock is the classification of hypotonic patients as patients with cardiogenic shock. This possibility is diminished by the fact that the same working unit examined the patients from one area employing the same criteria throughout all the series

The present material shows that the usefulness of medical treatment as a cure for cardiogenic shock is limited, particularly if myocardial infarction is the main etiology This is understandable in the light of the series of autopsies which show that 40–70% of the patients who die from cardiogenic shock have had extensive infarction (31 45). It seems that the whole basis of the pumping activity of the heart is lost in such cases. This is why various mechanical methods for supporting the circulation have been invented recently They have been used to reduce the work

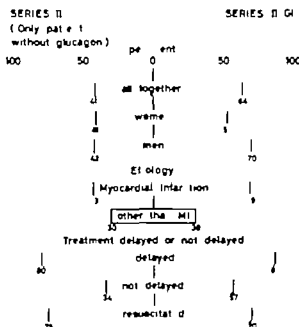


Fig. Mortahty in one eek in series II treated with and ithout glucagon.

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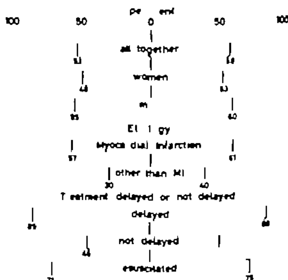


Fig 3 Comparison of the mortality rate in one week and in one month in series II.

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PTA (FACTOR XI) DEFICIENCY AND PROLONGED BLEEDING TIME

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Abstract. A 23-year-old man with an inherited haemorrhagic diathesis characterized by factor XI deficiency and prolonged Ivy bleeding time is reported The platelets aggregated normally with ADP but the disaggregation of platelets was rapid. The release of ADP induced by kaolin or adrenaline was defective, as was the release of PF 3 induced by kaolin. Thrombopathy was therefore diagnosed and the prolonged bleeding time was attributed to platelet defect. Administration of fresh platelets shortened the bleeding time. The level of factor XI was 1 5%. Although the administration of platelet-poor plasma corrected the coagulation defect, it had no effect on bleeding time.

Reports of a prolonged bleeding time associated with coagulopathy are rather rare except in patients with von Willebrand's disease. Association of prolonged bleeding time with a deficiency of factors IX (5 10 12), V (1) X (7) or XI (PTA) (11 23 24 32) has occasionally been observed. In von Willebrand's disease a plasmatic factor is responsible for the prolonged bleeding time (19), but in the other cases the mechanism of a prolonged bleeding time is not clear

A capillary defect has been reported in three cases of PTA deficiency associated with a prolonged bleeding time (11 23 24) In these cases, however the platelet functions were not studied in detail. White et al. (32) published a case of PTA deficiency associated with a prolonged bleeding time in which infusion of fresh platelets shortened the bleeding time and thereby suggested the presence of a qualitative platelet defect.

This paper reports a young man who had PTA deficiency associated with a prolonged bleeding time and normal platelet count and in whom platelet functions were studied in vitro and in vivo and found to be defective.

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MATERIAL AND METHODS

Collection of blood. Citrated venous blood as collected through 15-gauge needle emptying directly into siliconized tubes containing 1/10 vol. 3.8% trisodium citrate

Platelet-rich plasma (PRP) was prepared by centrifugation at 190 g for 10 min. *Platelet-poor plasma (PPP)* was prepared by centrifugation at 1 400 g for 20 min and *platelet-free plasma (PFP)* by centrifugation at 48 200 g for 30 min at +4°C.

The platelet count in PRP as always adjusted to 200 000/mm³ by addition of the patient own PPP in tests for determination of platelet aggregation and platelet factor 3 (PF 3) release. Studies on the platelet aggregation and the release reaction were completed within 2 hours of withdrawal of blood. Serum samples ere prepared as described previously (21).

Coagulation tests. Determinations of coagulation time in glass and plastic tubes, recalcification time of plasma, one-stage prothrombin time, prothrombin consumption, prothrombin + factor VII + factor X (Owren's P&P-test), factor V AHF (factor VIII), haemophilia B-factor (factor IX), tests for circulating anticoagulants, fibrinogen, thrombin time and fibrinolytic activity of plasma were performed by methods described earlier (21)

Factors XI and XII were determined by modifications of the methods described by Soulier and Prou-Wartelle (28) and Horowitz et al (10). The test base used consisted of normal PPP adsorbed with 30 mg celite/ml (Johns Manville, Lompoc, Calif.) for 10 min to remove factors XI and XII. The powder as removed by centrifugation of the mixture twice, each time at 4 000 g for 20 min. The supernatant plasma as adjusted to pH 7.5 with 0.1 N HCl or 0.1 N NaOH and incubated at 37°C for 6 hours, during hich it was frequently shaken. The platelet count in the plasma to be tested was adjusted to 200 000/mm³ before freezing. The assay was performed in the following ay: 0... ml of test base, 0 ml of Trombofax solution (Ortho) and 0.2 ml of the plasma to be tested in various dilutions was incubated for 4 min in new glass tubes in ater bath at 37°C, after hich 0.2 ml of 30 mM calcium chloride solution as added and the clotting time measured. The activity of factors XI and XII was expressed in relation to that found for normal standard consisting of pooled PRP (200 000 platelets mm³) from 10 normal individuals. Factor XII was assayed separately in an one-stage recalcification

system using plasma from patient with H geman trait (< 1% factor XII) as test base. (The Hageman deficient plasma was obtained from Dr Loeliger Holland.)

Thromboplastin generation test (TGT) was carried out by the method of Biggs and Douglas (3). Cephalin was used for platelet substitute in the TGT when the clotting defect was studied. PF 3 activity was measured in washed platelet suspension from the patient or normals; normal adsorbed plasma and normal serum were used. The distilled water test was carried out by the method of Ulutin and Karaca (30).

Platelet counts were made by the method of Björkman (4) or by Hellem modification (15) of Nygaard' method. The *bleeding time* was performed by Duke method using standardized haemolets (Dade Reagent Inc., Miami, Florida, USA), and by the method of Ivy as modified by Nilsson et al. (20).

Platelet adhesiveness as measured according to a slight modification (9) of H llem s whole blood method (15) and also by the original method of Salzman (27).

Clot retraction as measured by the method of Hartman and Conley (14) in plasma with decreasing concentrations of platelets.

Platelet aggregation studies. 1) Platelet adhesion, aggregation and spreading ere studied by phase contrast microscopy of a drop of PRP placed on glass slide unde a cover glass. These studies ere also performed after addition of thrombin and $CaCl_2$ solution to the PRP 7) Platelet aggregation as measured photometrically by the method of Born (6) with the use of an E.E.L. potentiometer linked to a UR 400 vitatron recorder (obtained from Prof G V R. Born, London). Aggregation studies ere performed at 37°C with the use of 1.8 ml samples of PRP in siliconized glass cuvettes. Constant stirring was maintained from below by plastic-coated magnetic tirrer rotating at 1200 rpm. White light was passed ough the tube, and the recorder was adjusted to give maximal optical density reading with PRP and full le deflection ith the corresponding PPP In the beginning, before addition of any aggregating agent, stir ring was continued for 1–2 min to obtain constant base time. Then 0.. ml of the aggregating agent was added to the cuvette and tracing as continued for 2–7 min, depending on the character of the tracing.

The aggregating agents were prepared as follows. The sodium salt f ADP (Sigma) and adrenaline acid tartrate BP were dissolved in distilled water to concentration of 1.0 mM and stored in 1 ml aliquots at – 20°C. Working solutions ere prepared from them by further dilution in barbital-buffered saline t pH 7.35 on the day they were to be used. ADP and adrenaline were used in final concentrations of 5 10^{-} M, 1 10^{-4} M and 5 10^{-4} M, respectively Human connective tissue extract (collagen) as prepared as described by Hovig (17). A suspension containing 0.6 mg nitrogen/ml was used as stock solution. The final concentrations of collagen used for aggregation were 1/40, 1/100 and 1/200 of the stock suspension.

The maximal slope was measured in mm/min for ADP and collagen-induced aggregation, as described by O'Brien et al. (22).

Platelet ADP release was studied either by stirring 0.9 ml PRP (> 300 000/mm³) with 0.1 ml collagen suspension (final concentration 1/40 dilution of the collagen stock solution) for 7 min at 37°C or by stirring 0.9 ml PRP with 0.1 ml 8% kaolin suspension for 15 min at 37°C or after addition of 0.1 ml thrombin (10 NIH U/ml) t 0.9 ml PRP for 10 min. After centrifugation the amount of ADP released into the supernatant was determined as platelet-aggregating equivalent, as described by Weiss (31). The results were expressed as 10^{-9} M ADP released per 10^9 platelets.

PF 3 availability was determined by the method of Hardisty and Hutton (13).

Release / PF 3 The method of Spaet and Cintron (29) as used for determining the kaolin-induced PF 3 release. In some tests ADP was added to the incubation mixture (0 1 ml ADP 10^{-4} M, 0.1 ml 5% kaolin suspension and 0.9 ml PRP). PF 3 activity as roughly estimated by interpolation of the clotting time on a standard dilution curve for normal PRP containing 200 000 platelets/mm³ and frozen and thawed 3 times. The value obtained for 200 000 platelets/mm³ was accepted as 100 %.

Platelet fibrinogen was determined by the method of Karaca et al. (18).

Case report

The patient, 23-year-old man, had since childhood had marked tendency to bruising and bleeding from the gingiva. H had had a prolonged bleeding after cuts and tooth extractions. After removal of adenoids in 1954 he bled excessi ly for several days, and the Hb fell to 5.9 g/100 ml. He had not been troubled by nose bleeding and had never had joint bleedings. Otherwise he as healthy and ell developed. The patient's father had had melaena on 3 occasions, but otherwise no bleeding symptoms. The mother had had no bleeding symptoms. He had one sister and one brother The 26-year-old brother had since childhood had marked tendency to nose bleeding, bleeding after cuts and also bruised easily The sister had no bleeding symptoms.

RESULTS

Results of routine haemostatic tests performed in the course of 2 years are given in Table I A prolonged Ivy bleeding time and prolonged coagulation time were constant findings. The coagula tion time in glass tubes was always over 4 min and in siliconized tubes it was over 50 min. The Ivy bleeding time was measured on 21 occasions and always found to be prolonged, viz. between 15 and 30 min. On the other hand the platelet count was always normal In addition the patient had a prolonged recalcification time and an abnormal prothrombin consumption test. The values for one-stage prothrombin time, P&P factor V and fibrinogen were normal. Factors VIII and IX were repeatedly normal. The clot retraction was also normal. The activity of factors XI and XII determined by modifications of the methods of

Table I. *Coagulation studies on the patient, his parents and brother*

	Patient: Range	Patient: No. of investigations	Father	Mother	Brother	Normal range
Bleeding time (min)						
Duke	3–4	10	1 3	1	2	1–4
Ivy	*13–30*	*21*	*12*	*9*	*10*	*6–12*
Coagulation time (min)						
Glass	*24–47*	24	14	15	25	8–14
Plastic	*30–80*	24	28	30	45	15–25
Prothrombin consumption (%)	*40–66*	17	38	13	25	0–30
Platelets/mm³	180 000–290 000	20	200 000	230 000	260 000	180 000–400 000
Platelet adhesiveness (%)						
Hellem's whole blood	*15–24*	7	49	41	37	18–32
Salzman's method	*15–41*	5	38	36	46	20–60
Clot retraction (residual serum, %)						
0.5 10⁶ platelets/mm³	68–70	2				65–75
0.25 10⁶ platelets/mm³	55–68	2				40–60
PF 3 availability (%)	*8–13*	4				90–150
Recalcification time (sec)	*280–400*	8	190	210	300	120–170
One-stage prothrombin time (sec)	14–16	4	17	17	16	14–16
P&P ([illegible])	85–110	4	108	99	91	80–120
Factor V (%)	83–100	4	83	100	105	80–120
Factor VIII ([illegible])	67 118	5	65	83	88	60–160
Factor IX (%)	133–145	2				60–160
Factors XI + XII (%)	*1 5*	18	*40*	*31*	[illegible]	60–160
Factor XII (%)	90	2				60–160
Fibrinogen (g/100 ml)	0.32	1	0.39	0.39	0 25	0 26–0 34
Fibrinolytic activity on fibrin plates (mm²)	33–70	2	30	0	30	0–70

Soulier and Prou-Wartelle (28) and Horowitz et al. (16) was determined on 18 occasions and varied between 1 and 5% of normal. The TGT was abnormal. Addition of either normal adsorbed plasma or normal serum corrected the defect in TGT. Assay of factor XII separately with Hageman deficient plasma as a test base gave a normal value. In addition the prolonged recalcification time of the patient's plasma became normal on addition of Hageman deficient

Table II. *Studies on platelet aggregation*

	ADP-induced aggregation (slope, mm/min) ADP final conc. (M)			Collagen-induced aggregation (slope, mm/min) Conc. of collagen stock solution			Adrenaline final conc. (M) 5 10⁻⁶		Platelet fibrinogen
	5 10⁻⁶	10⁻⁶	5 10⁻⁷	1/40	1/100	1/200	First wave	Second wave	(µg/10⁹ platelets)
Patient									
Mean	76.5*	14	0	107.5	34	10	Pos.	None	108
Range	70–83	12 16		85–130	—	—			
Normals									
Mean	65	33	19.5	77.6	66.3	38 6	Pos.	Pos.	184
Range	40–88	13–84	8–34	50–98	40–98	12–60			146–283
No. of determinations	10	15	11	11	10	9			12

* Disaggregation.

Table III *Studies on ADP and PF 3 releases*

	ADP release (10^{-9} M ADP/10^{8} platelets) induced by			PF 3 release (of PF 3 activity of normal PRP)		
	Kaolin	Collagen	Thrombin	Kaolin	ADP kaolin	Collagen
Patient						
Mean	4.3	20	13.2	10.7	70	7
Range	3.6–5	19.8–20.[illegible]	10.8–15.4	5–16.5	70–70	5–9
Normals						
Mean	16.7	17.8	17.8	50.7	86.4	21.6
Range	7.4–27.5	7.4–29.7	8.3–29	6.35–110	45–110	10–47
No of determinations	12	15	14	11	15	12

plasma, but not on addition of celite-adsorbed normal plasma. No anticoagulant activity could be demonstrated. The findings in this study thus suggested that the defective coagulation in this patient was due to factor XI (PTA) deficiency.

Platelet adhesiveness, as assayed with Hellem's whole blood method, was 70% (mean of 7 determinations) i.e. bordered the lower limit of the normal range. The mean value obtained by Salzman's method, was 26%. The appearance of the platelets in the phase contrast microscope was normal. In studies in Born's aggregometer the platelets aggregated normally after addition of ADP (Table II). But a rapid disaggregation was observed. There was no second wave on aggregation with adrenaline but collagen aggregation was normal.

When incubated with kaolin the patient's platelets released only an abnormally small amount of ADP. The release of ADP was, however, normal when the platelets were incubated with connective tissue and thrombin (Table III). The PF-3 release induced by kaolin was defective. Previous addition of ADP to PRP corrected the PF 3 release induced by kaolin. The platelets were also found to be defective in the TGT (Fig. 1). Incubation of platelets in distilled water corrected the defect in PF 3 activity. The fibrinogen content of the platelets was normal.

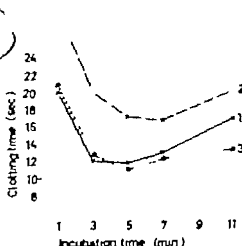


Fig. 1. Platelet factor 3 activity in thromboplastin generation test. *1* Normal adsorbed plasma + normal serum + normal platelets. *2* Normal adsorbed plasma + normal serum + patient platelets. *3* Normal adsorbed plasma + normal serum + patient platelets (after distilled water incubation). Platelets were tested at the concentration of 100 000/mm³

In order to clarify the mechanism of the prolonged bleeding time, the patient was given an infusion of concentrated fresh platelets in 150 ml of plasma (625 000 platelets/mm³) (Fig. 2). The Ivy bleeding time was shortened after infusion of the platelets and remained so for about 4 hours. The platelet aggregation with ADP and adrenaline, the ADP release after addition of kaolin, collagen and thrombin to PRP and the PF 3 release after addition of kaolin, did not noticeably change. Factors XI and XII increased from 5% to 13%. The coagulation time in glass and plastic tubes as well as the prothrombin consumption became normal and remained so for 48 hours. To assess the effect, if any, of a corresponding volume of plasma not containing platelets, the patient was later given 150 ml fresh PPP. It had no effect on the bleeding time, it increased factors XI and XII from 5% to 10% and shortened the coagulation time. The patient was later given 400 ml fresh plasma. The Ivy bleeding remained unchanged. Factors XI and

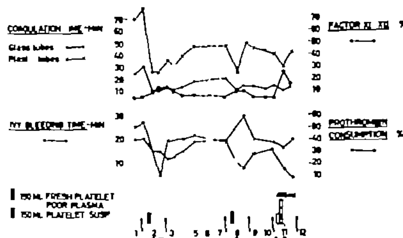


Fig 2 The effect of transfusion of platelets and fresh PPP to the patient. I, before II, 15 min after III, 90 min after infusion.

XII increased from 5 to 25% The results of various platelet function tests showed no improvement.

The father mother and brother of the patient were investigated (Table I) They all had a slightly prolonged coagulation time and recalcification time and low factors XI and XII, namely 40% 31% and 22% respectively They had normal bleeding times

DISCUSSION

The patient had since early childhood had symptoms of a rather severe haemorrhagic diathesis. All the coagulation studies indicated that he had factor XI deficiency The level of factor XI varied between 1 and 5% Both his parents also had decreased factor XI values, namely 40 and 31% The mode of inheritance of PTA deficiency is still debatable. According to Rosenthal et al. (26) the condition is autosomal dominant, while according to Rapaport et al. (25) the abnormality is transmitted by an autosomal recessive gene. Observations made in the present case argue for the latter mode of inheritance.

A remarkable observation in this patient was that the coagulation disorder was associated with a prolonged Ivy bleeding time. A prolonged bleeding time is generally caused by defect of the vessel wall, the platelets or plasma. In previous reports (11 23 24) a prolonged bleeding time was attributed to a capillary defect in three patients with PTA deficiency In one case (32) a platelet defect was observed.

Our patient showed a defective release of ADP induced by kaolin and adrenaline and a defective release of PF-3 and rapid disaggregation of the platelets. On the other hand the aggregation with ADP was normal. These findings are compatible with the presence of a release defect known as thrombopathy This type of bleeding disorder has been described by Weiss (31) Caen et al (?) Hardisty and Hutton (13) Their cases, however showed no signs of a coagulation defect. The thrombopathy in our case was only mild since there was no severe defect in ADP release induced by collagen. The prolonged bleeding time in our case could thus be attributed to a platelet defect.

Factor XI deficiency and defective PF 3 release resemble one another in some respects [illegible] defects may be more or less abolished by [illegible] of the plasma. Addition of kaolin activate [illegible] XI and releases PF 3. Biggs et al. (2) [illegible] cussed the coagulant activity of platelet [illegible] separated from PRP and incubated [illegible] hours at 37 C developed an activity [illegible] the coagulation time of plasma defic[illegible] XII, XI or VIII. This "coagulat[illegible] platelets might, perhaps, be identi[illegible] XI activity

However the transfusion [illegible] the factor XI deficiency in th[illegible] to the platelet defect. Infus[illegible] platelets suspended in 150 ml [illegible] by a shortening of the blee[illegible] tion time and by a slight [illegible] activity Infusion of PPP [illegible] on the bleeding time, [illegible] tion defect.

In the present cas[illegible]

[illegible] type[illegible] patients [illegible] ed con- Central [illegible] reported type IV [illegible] and 192

deficiency and mild thrombopathy must be considered.

The bleeding tendency both in PTA deficiency and thrombopathy is notably milder than that in haemophilia. The bleeding tendency in our case however was rather severe. This was probably due to the association of thrombopathy and PTA deficiency

ACKNOWLEDGEMENT

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CHANGE OF LIPOPROTEIN PATTERN BY CLOFIBRATE IN HYPERGLYCERIDAEMIA AND MIXED HYPERLIPIDAEMIA

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Abstract. The effect of clofibrate on plasma lipids, immunological β-lipoprotein and the electrophoretic (cellulose acetate) lipoprotein pattern has been studied in 13 and 27 patients with mild form of type IV and mixed hyperlipoproteinaemia (MH), respectively. Serum cholesterol and β-lipoprotein, which were correlated with each other only in type IV and which correlated negatively with relative b.wt. in type IV and positively in MH, were only slightly decreased by the drug. Thus the changes which were correlated positively with the initial values were insignificant for cholesterol in type IV and for β-lipoprotein in MH. Total triglycerides, which were correlated with pre-β-lipoprotein but not with relative b.wt., decreased equally (29%) in both groups, the decrement being correlated with the initial values. Lipoprotein electrophoresis revealed a relative fall of pre-β-lipoprotein and a relative increase of α-lipoprotein, the increment of the relative β-lipoprotein value being significant only in type IV in which the immunological β-lipoprotein was decreased. The change of the latter tended to correlate negatively with that of triglycerides in type IV and positively in MH. The results suggested that the serum lipid pattern is normalized by clofibrate in about one third of hyperglyceridaemic patients seen in the general population and that, owing to the rare occurrence of gross pre-β-hyperlipoproteinaemia, reciprocal increase of β-lipoprotein by the drug is seen infrequently.

A serum lipid lowering effect of clofibrate is seen most conspicuously in patients with hyperglyceridaemia (3, 4, 12, 23, 25) (type IV hyperlipoproteinaemia (1, 7)) though other hyperlipidaemic states with elevated triglyceride levels seem to respond to this drug too (7, 12, 13, 15, 23, 29); pure hypercholesterolaemia (type II A), particulary the familial form (30), is completely resistant to clofibrate. The marked reduction of triglycerides is associated with a clearcut decrease in very low density lipoproteins (VLDL, pre-β-lipoprotein) (6, 10, 12, 13) which transport most of the plasma triglycerides. On the other hand, observations of the clofibrate effects on cholesterol-carrying low density lipoproteins (LDL, β-lipoprotein) have given unequivocal results and LDL concentration may frequently increase (13, 14, 29). Since the high level of the latter has been generally accepted to predispose to development of coronary artery disease, the possible increase of LDL during long-term clofibrate therapy would be deleterious and may counteract the recently reported favourable effect of this drug on the prognosis of patients with angina pectoris (24). Therefore the effect of clofibrate on plasma lipids and different lipoprotein fractions, including immunologically determined β-lipoprotein, was investigated especially because the response to clofibrate may vary in different types of hyperglyceridaemia (29). For this purpose two hyperglyceridaemic groups, patients with pure type IV disease and with mixed hyperlipidaemia (MH) were treated with clofibrate and plasma lipids were analysed before and during therapy. Since the bulk of hyperlipidaemic patients in the general population suffer from the mild form of serum lipid abnormalities the patients of the two groups studied had only slightly or moderately elevated plasma lipid levels so that the results are applicable to most patients in general practice.

MATERIAL

The material includes 27 patients with MH (both hypercholesterolaemia and hyperglyceridaemia) and 13 patients with quite pure hyperglyceridaemia, type IV, treated consecutively for various reasons in the Kuopio Central Hospital. Clinical findings of the two groups, presented in Table I, showed that the patients with the type IV

Table I. *Clinical findings*

Am = aortic murmur, Cb = carotic bruit, Fb = femoral bruit, Xl = xanthelasmata, La = lipoid arcus, IHD = ischaemic heart disease, AO = obliterating arteriosclerosis of legs, CVA = cerebrovascular accident, D = diabetes

Sex[a] (♂/♀)	Age (y)	Weight (kg)	Weight (rel.)	Physical findings[a] Am	Cb	Fb	Xl	La	Total[b]	Diagnoses[a] IHD	AO	CVA	D
Hyperglyceridaemia (type IV) (n = 13)													
92/8	45±3	83±4	1.25±0.03	15	15	15	0	8	33	38	31	15	8
Mixed hyperlipidaemia (MH) (n = 27)													
85/15	47±2	74±3	1.16±0.03	48	11	26	15	22	69	52	41	0	15

[a] Per cent of patients in each group.
[b] Some of the findings present.
Statistically significant ($p < 0.05$).

abnormality were more obese but tended to exhibit physical signs of atherosclerosis less frequently than those with MH. The grouping of the patients was performed on the basis of the total cholesterol and triglyceride concentrations and lipoprotein electrophoresis as obtained during the first days of hospitalization. Thus the groupings apparently represent those occurring in free living conditions. The criteria of type IV disease were increased triglycerides (>2.0 <5.0 mmoles/l), normal serum cholesterol (<7.8 mmoles/l) and prominent pre-β band in the lipoprotein electrophoresis. The criteria of the MH group were elevated serum cholesterol (>7.8 mmoles/l) and triglyceride (>2.0 <5.0 mmoles/l) levels and prominent but clearly separated pre-β band in the lipoprotein electrophoresis. This group obviously comprised type II B hyperlipoproteinaemia (1), though no exact classification was possible. Immunologically determined β-lipoprotein was equal in the two groups, the clinical picture and family history indicated that there were no patients with type III hyperlipoproteinaemia and familial hypercholesterolaemia. After discharge from the hospital the patients were advised to take their usual home diet and they were re-examined in the Outpatient Department 3–4 months later. Analysis of the lipid status, as obtained from one to two blood samples in each patient, showed that, though quantitative changes had occurred, the pattern of total lipid components and lipoproteins was essentially unchanged. Thus no regrouping was performed, though some patients no longer fulfilled the original criteria of the grouping, probably owing to changes of dietary habits. Thereafter the patients were put on clofibrate 1.5 g/day (Atromid-S®, supplied by ICI, Pharmaceutical Division) for four to five weeks and serum lipids were reinvestigated at the end of the period. The values presented are means of two blood specimens drawn about one week apart. No consistent difference was seen between the two values, suggesting that blood lipids had already levelled off on a new low level. The body wt. remained unchanged during the treatment period.

METHODS

Serum total cholesterol was measured by the Liebermann-Burchard procedure (11) and triglycerides by combining

Table II. *Effect of clofibrate on serum lipids and lipoproteins in hyperlipidaemic patients*

Treatment	Serum lipids (mmoles/l) Chol.	Triglyc.	β-lipoprot. (g/l)	Lipoprotein electroph. (%) α	pre-β	β
Hyperglyceridaemia (type IV)						
None	7.3±0.3	3.49±0.24	8.0±0.4	14±1	34±2	52±2
Clofibrate	6.8±0.3	2.49±0.28	7.0±0.5	19±2	23±3	57±2
Change (mmoles/l)	−0.7±0.4	−1.00±0.29	−1.0±0.5	5±2	−11±3	5±2
Change (%)	−9±5	−28±7	−13±5	—	—	—
Mixed hyperlipidaemia (MH)						
None	9.1±0.3[a]	3.04±0.25	8.4±0.4	15±1	24±2[a]	60±2[a]
Clofibrate	8.0±0.2[a]	2.06±0.16	8.2±0.4	19±1	17±1	63±1
Change (mmoles/l)	−1.1±0.3	−0.98±0.17	−0.2±0.4	4±1	−7±2	3±2
Change (%)	−11±3	−29±4	−1±5	—	—	—

Statistically significant changes ($p < 0.05$).
[a] Statistically significant difference from type IV.

Table III. *Correlation between serum lipids, relative b.wt and lipoprotein fractions in hyperlipidaemic patients prior to and during clofibrate treatment*

B = before, D = during treatment

	Total cholesterol (mmol/l)		Total triglycerides (mmol/l)		Immunol. β-lipoprot. (g/l)		Rel. β-lipoprot. (%)		Rel. pre-β-lipoprot. (%)	
Subject	B	D	B	D	B	D	B	D	B	D
Hyperglyceridaemia (type IV)										
Rel. b.wt.	−0.56	−0.07	0.40	0.07	−0.74	−0.15	−0.66	−0.13	0.50	0.20
Immunol. β-lipoprot.	0.71	0.89	0.38	0.52	—	—	0.48	0.09	0.02	0.36
Rel. β-lipoprot.	0.20	−0.22	−0.47	−0.86	0.48	0.09	—	—	—	—
Rel. pre-β-lipoprot.	−0.22	0.56	0.74	0.9**	0.02	0.36	—	—	—	—
Mixed hyperlipidaemia (MH)										
Rel. b.wt.	0.67*	0.42	0.23	0.42	0.05	0.07	0.27	0.05	−0.02	0.06
Immunol. β-lipoprot.	0.25	0.66	−0.17	0.37	—	—	0.03	−0.09	0.01	0.47
Rel. β-lipoprot.	0.32	0.06	−0.42*	−0.21	0.03	−0.09	—	—	—	—
Rel. pre-β-lipoprot.	−0.01	0.16	0.69	0.65	0.01	0.47	—	—	—	—

Statistically significant correlations ($p < 0.05$).

the methods of van Handel and Zilversmith (9) and Sardesai and Manning (26). Lipoprotein electrophoresis was performed on cellulose acetate (5). The presence of chylomicrons in all turbid sera was checked by the agarose electrophoresis. However in none of the samples were chylomicrons found. Scanning of the cellulose acetate paper strips, stained with oil red O, was performed on Beckman Densitometer, Model 110. Separation between pre-β and β peaks was satisfactory in most cases. Relative quantities (% of total area) of β pre-β- and α-lipoproteins were calculated from the curve obtained from the scanning. Serum β-lipoprotein was quantitated by the immunodiffusion technique (2), originally designed for the immunodetermination of proteins by Mancini et al. (17). Serum of healthy subject with normal cholesterol (5.7 mmoles/l), low triglycerides (1.3 mmoles/l) and normal lipoprotein electrophoresis was used as control. Contribution of pre-β and α-lipoproteins to the immunological β-lipoprotein values was not studied.

RESULTS

Initial values. Serum cholesterol was significantly higher in MH than in type IV disease, yet immunological β-lipoprotein was of the same magnitude (Table II) suggesting that in the former abnormality β- or pre-β-lipoproteins contained some extra cholesterol or that pre-β-lipoprotein contributed to the β-lipoprotein values. No correlation was found between immunological β-lipoprotein and total cholesterol in MH, while th positive correlation in type IV (Table III) suggested that the bulk of the total serum cholesterol was associated with immunological β-lipoprotein.

The electrophoretic lipoprotein pattern revealed, as expected, the presence of a relatively low pre-β and high β in MH as compared to type IV (Table II) Relative β-lipoprotein values showed no correlation with total cholesterol levels, while a significant association was found between pre-β and total triglycerides in both groups (Table III). It is interesting to note that relative weight cor related negatively with serum cholesterol, immunological β- and relative β-lipoprotein in type IV this correlation being positive, if anything, in MH.

Effect of clofibrate Serum cholesterol decreased 10% or more in 62% of the patients with type IV and 52% with MH the average lowering (9 and 11%) being statistically significant in the latter group only (Table II) The mean decremets of the two groups were not significantly different, however and 60% of the mixed hyperlipidaemics still remained hypercholesterolaemic after clofibrate treatment for one month. Serum triglycer ides fell 10% or more in 85% of the patients in both groups, the mean reduction being equal, viz. 28 in type IV and 29% in MH, yet about half of the patients were still hyperglyceridaemic. Both cholesterol and triglycerides were normalized in 38 and 33% of the patients in type IV and MH, respectively

Despite the insignificant fall in the total serum cholesterol, the immunological β-lipoprotein concentration decreased significantly in type IV

(−13%) but remained unchanged in MH in which the fall in total serum cholesterol was significant (Table II) The change of immunological β-lipoprotein correlated, however with that of cholesterol in both groups ($r = 0.91$ and 0.55 in type IV and MH respectively) It was owing to a marked absolute fall in pre-β-lipoprotein and only a small absolute fall in β-lipoprotein that the relative amount of the latter tended to increase (Table II) The correlation between the changes in immunological β-lipoprotein and total triglycerides (reflects the amount of VLDL) tended to be negati e in type IV ($r = -0.61$) and positive in MH ($r = 0.31$) The relative increase of α-lipoprotein was considerable, though calculations suggested that its absolute increase was negligible.

Disappearance of the negative correlation between relative b.wt. and total cholesterol or β-lipoprotein in type IV during clofibrate treatment (Table III) suggested that the drug was more effective in the lighter than heavier patients, owing to the higher relative dose of the drug, or that the higher initial values were reduced more effectively than the lower ones. In favour of the latter alternative are the findings that the absolute and relative change of total cholesterol (and β-lipoprotein) showed a positive correlation with the initial values not only in type IV ($r = 0.64$) but also in MH ($= 0.64$) The same to triglycerides ($= 0.55$ and 0.77 for the solute change in type IV and MH respectively) In MH the immunological β-lipoprotein alues correlated during treatment in contrast to pre treatment values, with total cholesterol (Table III) suggesting that most of the total serum cholesterol was now transported by immunological β-lipoprotein.

DISCUSSION

The present findings showed that in the two quite distinct types of hyperglyceridaemia, type IV and mixed hyperlipidaemia, serum triglyceride levels fell equally cholesterol reduction being negligible, so that normal lipid levels were obtained in about 35% of all patients. Since the material included only patients with mild or moderate hyperlipidaemia, the lipid abnormality seen most commonly in the general population, it may be concluded that clofibrate alone, without weight reduction or any other dietary manipulations, normalizes serum lipids in one third of hyperglyceridaemic patients living freely in modern society

Immunologically determined β-lipoprotein, the response of which to clofibrate has not been reported earlier was slightly reduced or remained unchanged. In lipaemic type IV patients a marked clofibrate-induced fall of VLDL is associated with an increased LDL, possibly owing to enhanced conversion of VLDL to LDL, while this type of reciprocity is not seen in mixed unclassifiable hyperlipidaemia or type III hyperlipoproteinaemia (15 29) In the present series, which included only mildly to moderately elevated values, no such increase of LDL was seen in the type IV patients, though the change of immunological β-lipoprotein tended to correlate negatively with that of triglycerides (reflects mostly VLDL) in type IV and positi ely in mixed hyperlipidaemia. Thus, because clofibrate quite unquestionably reduced pre β-lipoprotein (VLDL) and decreased β-lipoprotein in almost half of all patients, the drug appears to have a beneficial effect on the lipoprotein pattern in mild and moderate hyperglyceridaemia (type IV and mixed hyperlipidaemia).

Most of the patients of the present series, especially those with type IV disease, were obese. Though the relative weight has been shown to correlate with both serum cholesterol and triglycerides (*16 18 22*) the type of *hyperlipidaemia* seems to be important in this respect because cholesterol and β-lipoprotein correlated in the present study negatively with b.wt in type IV and positively in the mixed hyperlipidaemia. A frequent occurrence of type IV abnormality in the general population is thus apparently one reason why the correlation between serum cholesterol and b wt. is poor in many epidemiological studies (22)

Obesity strongly stimulates cholesterol production in man so that the excess weight correlates with the excess production (19). Therefore most patients in this study quite apparently had endogenous overproduction of cholesterol. However despite markedly enhanced synthesis, serum cholesterol is suggested to remain low in pure type IV disease because of very effective elimination of cholesterol from the body (20 21). Thus the type IV patients of this series, especially the most

obese, had the high triglyceride levels, excessively produced cholesterol being apparently excreted effectively into the stools as bile acids and neutral sterols, so that serum cholesterol remained normal. In the mixed hyperlipidaemia group, on the other hand, hypercholesterolaemia and probably hyperglyceridaemia, too, may have developed primarily as a result of removal defect (20, 21). It thus seems apparent that correction of the latter in mixed hyperlipidaemia would rapidly normalize blood lipids, while inhibition of synthesis in pure type IV would be preferable. The exact mode of action of clofibrate is not yet known. Its relatively poor effect on serum cholesterol and β-lipoprotein suggests that the drug quite weakly stimulates cholesterol elimination and catabolism of β-lipoprotein, nor does its possible inhibition of synthesis of those parameters appear to be very strong. In view of this fact it is important to note that, in man, clofibrate has been reported to augment the elimination of cholesterol into faeces and to inhibit cholesterol synthesis (8, 28). The reduction of plasma triglycerides by clofibrate has been suggested to be due to an increased triglyceride removal (27). The finding that only about one third of the relatively mildly hyperlipidaemic patients were rendered normolipidaemic by clofibrate in the present study indicates that lowering of lipid production and stimulation of elimination by weight reduction and other dietary measures should be combined with clofibrate treatment.

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DETERMINANTS OF THE RESPONSE TO COUMARIN ANTICOAGULANTS IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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Abstract Determinants of the response to coumarin anticoagulant, warfarin, have been analysed in series of 19 patients with acute myocardial infarction. Cardiac failure developing in the acute phase of the disease seemed to be without any great influence on the plasma half-life of warfarin, with the exception of patients in whom serious circulatory catastrophes occurred. In patients who had received other drugs regularly before the hospital admission the plasma half-life of warfarin showed significant prolongation within 3 weeks—a phenomenon apparently caused by decreasing induction of microsomal drug-metabolizing enzymes in the liver. The plasma half-life of warfarin was found to explain 44% of the variance in the daily requirement of warfarin during maintenance treatment. The steady state "therapeutic" plasma warfarin level showed wide range of individual variation—from 0.8 to 4.1 mg/l, with median value of 1.5 mg/l. The results indicate that in addition to the rate of coumarin anticoagulant metabolism other factors have marked influence on the response to coumarin drugs in patients with myocardial infarction.

It is well known that patients in whom a severe cardiac failure develops in connection with acute myocardial infarction (AMI) often show an increased sensitivity to coumarin anticoagulants, especially during the first week after the acute attack. An impaired synthesis of prothrombin complex clotting factors apparently is an important factor accounting for this increased sensitivity. In patients with severe myocardial infarction plasma levels of these clotting factors may decrease spontaneously as an indication of a disturbance in the function of the liver cells. "Liver-specific" serum enzymes, LDH_5-isoenzyme and OCT often show a rise in cases of severe myocardial infarction, although these changes are not necessarily related to the degree of impairment of all aspects of liver function (1 3 4). Microsomal drug-metabolizing enzymes of the liver cells are responsible for the biotransformation of coumarin drugs. A decrease in the capacity of this enzyme system has been demonstrated in parenchymatous liver diseases (9). So far it is not known to what extent the disturbance of liver function developing in cases of AMI with severe cardiac failure affects the ability of the microsomal enzymes of the liver to metabolize drugs.

In the following we report results of a study on the determinants of the coumarin anticoagulant response in patients with AMI with special emphasis on the possible effects of cardiac failure developing after the acute attack on the rate of metabolism of the coumarin drug, warfarin. Because the medication patients had received before admission to the hospital is an important confounding variable in studies of this kind, the results were also analysed with regard to its effects. Correlation of the plasma half-life of warfarin to the average daily warfarin requirement during stable anticoagulant effect was analysed and the range of individual variation in the steady state "therapeutic plasma warfarin level was determined to obtain information about the extent to which other factors than the rate of drug metabolism determine the response to coumarin anticoagulants in patients with AMI.

MATERIAL AND METHODS

The series comprised 19 patients, 11 men and 8 women, with AMI. The mean age of the patients was 61.2 years (range 39–79 years). On the basis of ECG findings the infarction was transmural in 11 and subtransmural in 8 cases. The following three grades were used in the classification of the series according to the degree of cardiac failure developing within 24 hours since the

Table I. *Drugs used by the patients with "significant" pre-hospital medication in whom the plasma half-life was determined on admission and 3 weeks later*

Pat. no.	Drugs
1	Diazepam, Bronkelosyl[a], nitroglycerin
2	Chlordiazepoxide, reserpine, prenylamine, furosemide, lanatoside C
3	Theobamat[b], nitroglycerin
4	Indomethacin, α-methyldopa
5	Myangin[c], glyphylline, digoxin, nitroglycerin
6	Myangin, isoniazid, digoxin, nitroglycerin

[a] Contains ephedrine hydrochloride, choline theophyllinate and guaiphenesin.
[b] Contains meprobamate, theophylline, papaverine hydrochloride, atropine sulphate and pentaerythritol tetranitrate.
[c] Contains phenobarbital, glyphylline, papaverine hydrochloride, atropine sulphate and nitroglycerin.

admission to hospital: *Grade I.* No clinical evidence of cardiac failure (6 pats.). *Grade II.* Mild or moderate cardiac failure (6 pats.). *Grade III.* Severe cardiac failure (7 pats.).

Two patients having a grade III cardiac failure showed, in addition to left heart failure, clear-cut signs of right heart failure, as revealed by peripheral oedema and enlargement of the liver. Patients with cardiogenic shock were excluded, because it was impossible to carry out the study program in these severely sick patients.

In two patients with a grade III cardiac failure, cardiac arrest due to ventricular fibrillation developed within 24 hours after admission. In one of them sinus rhythm was restored within a few minutes with a single shock and the patient thereafter made an uneventful recovery. The other patient was resuscitated twice from cardiac arrest and had several attacks of ventricular tachycardia treated with DC shocks between these two cardiac arrests. In spite of these severe complications he also finally made a good recovery. On the 2nd day in hospital one patient with grade III cardiac failure developed atrial fibrillation with a ventricular response of about 150 beats/min, which lasted for 4 days, whereafter sinus rhythm was restored by digoxin treatment.

All drugs used by the patients within 3 months before admission were recorded. Because of the great variety of drugs used the series was simply divided into two groups: patients without "significant" pre-hospital medication (13 pats.) and with "significant" pre-hospital medication (6 pats.). "Significant" medication means that the patient had used some drug(s) more or less regularly. An occasional use of drugs without known interference with the action of other drugs was not taken into account.

Table I lists the drugs used by 6 of 14 patients in whom the half-life of warfarin could be determined twice. Among these patients, in whom the half-life of warfarin could be determined only at the beginning of the treatment, were 3 who had used drugs (lanatoside C and nitroglycerin in 1 case, Myangin®—a combination preparation—in 1, and digoxin, furosemide, nalidixic acid and sulfafurazole in 1). The drug treatment in hospital, given in addition to anticoagulant therapy was, as far as possible, limited to analgesics needed in the acute phase, to drugs needed in the treatment of cardiac failure or arrhythmias, and to drugs needed for sedation in acute phase. Barbiturates were avoided and diazepam was the sedative mainly used. However some patients received also other drugs, as shown in Table II, listing all the drugs used in the treatment of the 19 patients. As the list indicates, antibiotics and chemotherapeutics had to be given to some patients to control concomitant infections. The drugs at the end of Table II are mainly components of combination preparations which the patients received temporarily at the order of attending physician.

For the determination of the half-life of warfarin in the plasma, the patients were given a standard oral dose of warfarin sodium, 0.7 mg/kg b.wt., in the fasting state. Venous blood samples were taken before the administration of the test dose and 12, 24, 36 and 48 hours after it. The method described by O'Reilly et al. (11) was used for the determination of the warfarin concentration in plasma. The half-life of warfarin was determined from the disappearance curve of the drug plotted on semilog scale. In 14 patients the half-life of warfarin in plasma was determined twice. The first determination was carried out soon after admission and the second 3 weeks later. At the time of the second warfarin half-life determination cardiac failure was well compensated in all cases. Administration of daily maintenance doses of warfarin

Table II. *Drugs administered to the 19 patients in hospital*

Drugs[a]	No. of pats. receiving each drug
Analgesics	
Morphine	10
Pethidine	7
Pentazocine	2
Codeine	1
Antiarrhythmic drugs	
Lidocaine	6
Procainamide	4
Atropine	3
Practolol	1
Cardiac glycosides	
Digoxin	13
Diuretics	
Furosemide	11
Antibiotics and chemotherapeutics	
Ampicillin	2
Streptomycin	1
Tetracycline	1
Nalidixic acid	1
Sulfafurazole	2
Nitrofurantoin	2

[a] In addition to the drugs listed, the following drugs were temporarily given, each of them to one patient: glyphylline, xylometazoline, isoniazid, pheniformin, propantheline, bromhexine, nicotinic acid, meclizine, hydroxyzine, ephedrine.

as begun after the initial determination of the half-life of warfarin. The daily doses of warfarin were always given at 8 p.m. Maintenance treatment as stopped 5 to 7 days before the second warfarin half-life determination. In 4 of the 19 patients only the *first* determination of the half-life of warfarin could be carried out.

The P&P (prothrombin plus proconvertin) method of Owren and Aas (13) was used in the control of anticoagulant therapy and the aim as to keep the P&P level within the range of 10-20%.

In 15 patients blood samples were collected before the administration of daily maintenance doses of warfarin *for the determination of the "therapeutic" plasma warfarin* concentration after the 5th day when the P&P level had settled within the therapeutic range. The results of at least t determinations of plasma warfarin concentration during 5-day period of stable anticoagulant effect ere used in the calculation of the "therapeutic" plasma warfarin concentration. The average daily requirement at the time of the determination of plasma warfarin concentration was expressed in mg/kg b.wt.

RESULTS

Half-life of warfarin in plasma

In 12 patients with grades I or II cardiac failure the mean plasma half-life of warfarin on admission was 39.1 h (S.D. 13.8 h). In 7 patients with grade III cardiac failure the corresponding mean value was almost the same 37.1 h (S.D. 9.6 h). Fig. 1 shows the plasma half-life values on admission and 3 weeks later in 8 patients with grades I or II cardiac failure and in 6 patients with grade III. The change in the half-life of warfarin showed no systematic trend in these two groups. In two patients showing initially clear signs of right heart failure on admission the half-life of warfarin did not show any shortening, when the heart failure subsided. However a considerable shortening in the *half-life* of warfarin occurred in two other patients with grade III cardiac failure. One of them was the patient who on the 1st day was resuscitated twice from a cardiac arrest, and the other was the patient who on the 2nd day developed atrial fibrillation with a rapid entricular rate lasting for 4 days. When the changes of the plasma warfarin half-life in these two patients were compared with the mean change of the warfarin half-life in the other 12 patients, the differences were found to be highly significant ($p < 0.005$).

In Fig the results are presented by dividing the patients into two groups in regard to the *presence or absence of a significant pre-hospital*

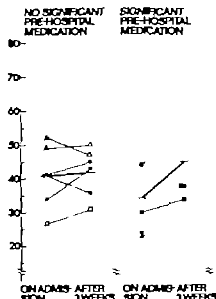


Fig. 2. The changes in the half-lif of arfarin in plasma in 6 patients without and 6 patients with significant pre-hospital medication. The mean values are indicated by short horizontal lines. Symbols as in Fig. 1.

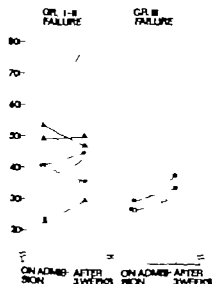


Fig. 1. The changes in the half-life of warfarin in plasma in 14 patients with AMI in relation to the degree of cardiac failure on admission. Grade I: ○ = without, ● = with significant pre-hospital medication, grade II: △ = without, ▲ = with significant pre-hospital medication, grade III: □ = without, ■ = with significant pre-hospital medication.

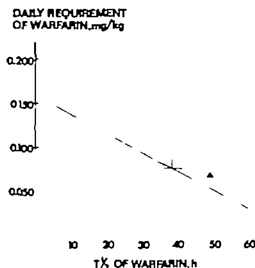


Fig. 3 The correlation between the plasma half-life of warfarin and the daily requirement of warfarin in 15 patients with AMI. Regression equation: $y = 0.160 - 0.002x \pm 0.013$ $r = -0.66$; $p < 0.01$. Symbols as in Fig. 1

medication. In patients without prehospital medication the mean value for the half-life of warfarin did not show any definite change. In patients who had had pre-hospital medication the mean value for the half-life of warfarin showed a significant prolongation ($p < 0.01$)

Correlation between the plasma half-life of warfarin and daily warfarin requirement

As expected, the plasma half-life of warfarin showed a negative correlation to the subsequent daily warfarin requirement (Fig. 3). The two patients in whom the plasma half-life of warfarin was initially prolonged in connection with serious circulatory complications were excluded from this analysis. The correlation coefficient expressing the relationship between the plasma half-life of warfarin and warfarin requirement was −0.66. Consequently the half-life of warfarin explained 44% of the variance in the daily warfarin requirement.

"Therapeutic" plasma warfarin level

The "therapeutic" plasma warfarin level determined in 15 patients 24 h after the preceding warfarin dose showed a wide range of individual variation—from 0.8 to 4.1 mg/l with a median value of 1.5 mg/l. Patients with similar values for plasma half-life of warfarin showed about fourfold differences in the daily warfarin requirement and also differences of similar magnitude in the "therapeutic" plasma warfarin level. The grade of cardiac failure showed no definite relationship to the "therapeutic" plasma warfarin level.

DISCUSSION

Cardiac failure developing as a consequence of AMI may result in a decrease of liver blood flow and in a congestion of the liver. These circulatory changes do not always seem to have any great effect on the rate of metabolism of coumarin anticoagulants by the liver cells. However in the present study circulatory catastrophes caused by serious arrhythmias were found to cause a prolongation of the plasma half-life of warfarin. Coumarin anticoagulant metabolism may also be slowed down in cardiogenic shock, although it was not possible to study it, because the condition of a patient with cardiogenic shock precludes the administration of a large initial dose of warfarin. In any case, the results of the present study support the view that, with the exception of the most severe instances of circulatory failure, an increased sensitivity to coumarin anticoagulants in patients with AMI is not due to a retardation of the metabolism of these drugs. It is apparently mainly explained by an impairment of the synthesis of vitamin-K-dependent clotting factors in the presence of liver congestion. A temporary reduction of dietary vitamin K supply due to restricted food intake may also contribute to increased anticoagulant sensitivity in the acute phase of the disease.

In the present series 6 of the 14 patients in whom the plasma half-life of warfarin was determined twice had received various other drugs before admission to hospital. Phenobarbital, a potent inducer of the drug-metabolizing enzymes, had been used by two patients, and meprobamate, also a known inducer (5) was included in the medication of one patient. Furthermore, chlordiazepoxide and diazepam were included in the list of drugs taken by the patients. These compounds are often mentioned as potential inducers, since they have been found to cause induction of drug metabolism in the rat (6, 8) We have shown that diazepam causes an induction of war-

farin metabolism in the rat, but we failed to demonstrate any consistent shortening in the plasma half-life of warfarin in humans, although the diazepam dosage used was as high as 30 mg daily (15). Some patients in the present series had received other drugs, like α-methyldopa, indomethacin and prenylamine, the effect of which on the metabolism of other drugs is unknown. In this heterogenous group of 6 patients having pre hospital medication the plasma half-life of war farin showed a significant prolongation within 3 weeks. The most likely explanation is that, at least in some of these patients, the drug-metabolizing enzymes had been induced by the pre-hospital medication and that the elimination of the drugs used at home from the regimen resulted in the retardation of drug metabolism.

The other possibility that must be considered is that drugs given during hospital treatment soon after admission might have interfered with the binding of warfarin to plasma albumin and shortened the half-life of warfarin in plasma. Seven of the 15 patients in whom the half-lif of warfarin was determined twice received furosemide. A non-diuretic thiazide, diazoxide, has been shown to displace warfarin from its albumin binding (17). Since furosemide is closely related to thiazides, it also might have similar properties. However in the present series the use of furosemide had no relationship to the changes in plasma warfarin half-life.

If a sedative was needed in the acute phase treatment of our patients we chose diazepam, because it seems to have no definite effect on coumarin anticoagulant metabolism in humans, as mentioned above. Of the analgesics used in the acute phase treatment morphine and pethidine have been found to potentiate the effect of coumarin anticoagulants in animal experiments (7) and, indeed, we have found that pethidine retards the rate of warfarin metabolism in the rat (16). According to our preliminary observations pethidine in the dosage used clinically may cause some prolongation in the plasma half-life of warfarin in humans. As regards lidocaine and procainamide given for the treatment of ar rhythmias, nothing is known about the possible interference of these drugs with the metabolism of coumarin anticoagulants.

The rate of metabolism of a given drug in each individual naturally is an important determinant of the dosage of the drug required to maintain the therapeutic effect. In the present series the rate of warfarin metabolism expressed as half life of the drug in plasma explained 44% of the variance in the maintenance dosage of warfarin. In accordance with previous findings of others (2) and ourselves (14) the "therapeutic" plasma war farin level during maintenance treatment showed a wide range of individual variation.

Several factors may account for the wide range of variation in the "therapeutic plasma warfarin level. In some patients with low "therapeutic plasma warfarin levels the capacity of the liver cells to synthetize prothrombin complex clotting factors may be subnormal due to liver congestion or pre-existing liver disease. Individual differences in vitamin K balance and in the amount of vitamin K available at the receptor sites for coumarin drugs may in part account for the individual differences in the sensitivity to coumarin drugs. It has been maintained that individual differences in the affinity of the receptor sites for coumarin drugs might also be responsible for differences in the response to coumarin anticoagulants (18). Although the existence of such individual differences in the affinity of receptors for coumarin drugs and, also for vitamin K cannot be denied, these are so far hypothetical, with the exception of the rare inherited resistance to coumarin anticoagulants in which the anomaly has been located at the receptor site (10, 12)

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CLINICAL AND RADIOLOGICAL SIGNS OF LEFT VENTRICULAR FAILURE IN ACUTE MYOCARDIAL INFARCTION

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Abstract. T analyse the value of clinical signs and chest X-ray parameters in the diagnosis of left ventricular (LV) failure in proven acute myocardial infarction correlative study has been performed in 110 patients during the first three days of hospitalization, whilst in the Coronary Care Unit. The clinical signs assessed were: 1) third heart sound, 2) basal crepitations, mild and marked, and 3) frank pulmonary oedema. The radiological parameters indicative of LV failure were: 1) pulmonary vessel (venous) dilatation, moderate and marked, 2) pulmonary oedema, patchy and diffuse, 3) Kerley' (septal) lines, and 4) pleural effusion. The incidence of LV failure on the first three days, if clinical signs only were utilized, as 31%, 30% and 14% respectively If radiological signs only ere used, the incidence of LV failure on days 1, and 3 was 56%, 43% and 29% respectively If both clinical and radiological findings were used, the incidence of LV failure on days 1 2 and 3 was 60%, 48% and 34% respectively A total of 45 of the 110 patients (41%) showed clinical evidence of LV failure, and 63 (57%) showed radiological evidence of LV failure. These figures do not include the patients ho died shortly after admission, before chest X-ray could be taken. The chest X-ray proved to be more sensitive in the detection of LV failure, and it also detected LV failure earlier LV failure was detected in the chest X-ray one day earlier than by clinical signs, in 17% of these patients with LV failure. Intra-alveolar pulmonary oedema, shown in the X-rays, was not associated th clinical signs in 41% of cases. The earliest radiological parameter of LV failure was pulmonary vessel dilatation, hich gives rise to no auscultatory signs.

The presence of left entricular (LV) failure in acute myocardial infarction (AMI) is associated with an increased mortality (14 16) and thus early diagnosis and treatment are desirable. The detection and assessment of LV failure are for routine purposes based on clinical signs and chest X-ray findings.

More direct methods for detection and assessment of LV failure in AMI, such as measuring the pulmonary artery wedge pressure and also the left heart pressure, are reported (2, 8 9 16). The routine use of these methods, however is not yet practical in most intensive care units, except under special circumstances, and these procedures, particularly left heart pressure measurements, are not without risk in patients with AMI.

To the authors knowledge ery little has been published correlating clinical signs and chest X ray findings of LV failure in AMI (16, 17). It has been suggested that chest X-rays may be of more value on some occasions, in the detection of LV failure, the chest X-rays being indicative of LV failure in the absence of clinical signs (3 10 14)

To evaluate the relationship between clinical signs and chest X-ray findings of LV failure in AMI a correlative study was performed.

MATERIAL AND METHODS

Patients

From Sept. 1969 to Jan. 1971 172 patients were admitted to the Coronary Care Unit (CCU) with the preliminary clinical diagnosis of AMI. These patients were reviewed retrospectively in order of admission. The patients ages ranged from 36 to 82 years, mean 66 years.

The clinical diagnosis of AMI as considered established if one of the following criteria as present.

1. Pathological Q waves, accompanied by elevation of the S-T segments and subsequent inversion of the T waves.

2. S-T segment elevation and subsequent T wave inversion, accompanied by significant transient rise in serum SGOT (40 U) and serum LDH (400 U)

3. Left bundle branch block, accompanied by similar enzyme changes.

4. Post mortem evidence of fresh infarction in patients ho died on the day of admission.

Twenty-six patients failed to meet these requirements

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Fig. 1. A 55-year-old man with AMI. (*a*) Day 1. Marked pulmonary vessel dilatation. (*b*) Day 2. Moderate pulmonary vessel dilatation.

and were excluded from the study. Fourteen patients, although meeting the requirements for the diagnosis of AMI, were subsequently found to have inadequate clinical notes, and were also excluded. The remaining 132 patients met the requirements for the diagnosis of AMI and had full clinical notes.

Twenty-two patients were excluded because of incomplete radiological data. In 9 patients X-rays were mislaid, and in 13 death occurred prior to the chest X-rays being taken (the majority of these patients had frank pulmonary oedema). Thus the total number of patients in the study was 110.

The latent period between the clinical onset of myocardial infarction and admission to the CCU was 2 days for 2 patients, 3 days for 6, 4 days for 1 and 5 days for 4 patients. The remaining 97 patients were admitted within 24 hours.

Twenty-one patients had suffered previous infarction. Twenty-five patients had been treated prior to admission with digitalis and diuretics for congestive heart failure, associated in the majority of cases with ischaemic heart disease. Eleven patients were under treatment for hypertension, 14 for diabetes, and 2 were known to have obstructive lung disease. As a rule most patients remained in the CCU for 72 hours. Ten patients remained longer due to complications, and 9 were transferred to the main ward after 48 hours due to pressure of admissions to the CCU. The routine treatment for LV failure was in the majority of cases furosemide and digitalis.

Clinical and Radiological Methods

Complete clinical and radiological data concerning the presence or absence of LV failure were available only during the period of management in the CCU which, as previously stated, was in most cases 72 hours. For this reason this study was confined to the first 3 days of hospitalization, while the patients were in the CCU.

Clinical signs of LV failure

The presence or absence of clinical signs was assessed daily on the morning rounds. The clinical signs assessed were: 1) third heart sound, 2) basal crepitations, mild and marked, and 3) frank pulmonary oedema (frothy sputum, generalized crepitations). The presence or absence of these signs was recorded on a special data sheet, which was used throughout the period of the study. The assessments were made, in most instances, by the senior cardiologist in charge of the Department of Cardiology.

Unfortunately it was not possible to include the clinical signs on admission in the correlation study as the chest X-rays were not taken until after the morning round, except under special circumstances. However, the clinical findings on admission were reviewed retrospectively in the 110 patients in this study. It was found that in 7 patients clinical signs of LV failure were present on admission, but no longer on the morning round on day 1. It was also found that in 8 patients clinical signs were absent on admission, but present on the morning round on day 1. The exact time of admission was noted in the majority of patients, so enabling determination of the period of delay between the time of admission and the morning round on day 1. In 75% of the patients the delay period was 10–20 hours, mean 14 hours (the 15 patients in whom there was a change in the clinical signs were in this group). In 25% of the patients the delay period was 1–9 hours, mean 4 hours.

Radiological parameters of LV failure

A bedside chest X-ray was taken after the morning round in all patients with AMI.

A portable X-ray apparatus was used. The film-focus distance was 150 cm and the exposure factors were 100 kV 200 mA and 0.03-0.04 sec. Cassettes with built-in grids with 8 : 1 ratio and highspeed-screens and -films were used. As a rule antero-posterior roentgen film was taken, with the patient' chest elevated 45° from the horizontal position. Great care was taken properly to position and centre the cassette with the grid, the patient and the central X-ray beam. For this purpose water levels with scales placed both on the cassette and on the X-ray tube proved to be of considerable value.

The quality of the bedside X-rays was good in most patients, and in all instances adequate for the purposes of this study. The time delay between the clinical assessment and the chest X-rays was short, ranging from 1 to 4 hours.





Fig. 2. A 74-year-old man with AMI. (*a*) Day 1. Patchy pulmonary oedema, but no clinical signs of LV failure. (*b*) Day 2. Diffuse pulmonary oedema.

The chest films were reviewed retrospectively by the senior radiologist in charge of thoracic radiology in conjunction with a member of the staff. The radiologists had no knowledge of the clinical findings with respect to LV failure.

The parameters noted and considered to be indicative of LV failure were: 1) pulmonary vessel (venous) dilatation, moderate and marked (Fig. 1), 2) pulmonary oedema, patchy and diffuse (Fig. 2), 3) Kerley' lines, and 4) pleural effusion.

The heart size was recorded, but not used as a parameter of LV failure. Mild pulmonary vessel dilatation was not included, as a high degree of observer error is likely and it is difficult to differentiate from the normal; therefore its significance as a parameter of LV failure is considered to be uncertain.

Progress bedside chest X-rays obtained in 10 patients on the fourth day were reviewed, as were the standard chest films taken in the second and third week in the surviving patients. Unfortunately correlation with clinical signs was not possible, as the case notes during this period were incomplete. However the findings in the progress X-rays are included in the results, as they are significant with respect to the pattern of change of the radiological parameters of LV failure.

RESULTS

Clinical signs of LV failure

Thirty-four of the 110 patients had clinical signs of LV failure on day 1. On day 2 clinical signs had developed in 11 patients, and in 20 patients,

Table I. *Incidence of radiological parameters and clinical signs*

% = relative incidence, PV = pulmonary vessel dilatation, moderate (+) and marked (+ −), PO = pulmonary oedema, patchy (p) or diffuse (d), KL Kerley' lines, PEf = pleural effusion, BC = basal rales, moderate (+) and marked ( ), 3HS third heart sound

	Day 1		Day 2		Day 3	
No. of pats at risk	110		104		97	
	(*n*)	( )	(*n*)	( )	(*n*)	(%)
Radiological parameters						
PV + +	22	35	20	44	8	29
PV + + +	29	47	17	38	11	39
POp	15	24	8	18	7	25
POd	5	8	3	7	2	7
KL	15	24	7	16	5	18
PEf	18	29	16	36	14	50
No. of pats.	62		45		28	
Clinical signs						
BC +	17	50	13	42	9	64
BC	10	29	5	16	0	
3HS	14	41	18	58	8	57
No. of pats	34		31		14	

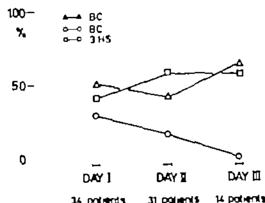


Fig. 3. Incidence of clinical signs of LV failure. Abbreviations as in Table I.

previously noted on day 1 clinical signs remained. On day 3 no further patients had developed clinical signs, and these signs remained in 14 patients previously noted on days 1 and/or 2. Thus 45 patients (41%) showed clinical evidence of LV failure. Forty four patients had basal crepitations and/or third heart sound and one patient had frank pulmonary oedema.

Fig. 3 and Table I show the relative incidence of clinical signs, basal crepitations, mild and marked and third heart sound, on days 1, 2 and 3 (34, 31 and 14 patients, respectively). (The patients with pulmonary oedema on day 1 are not shown.)

Radiological parameters of LV failure

indicative of LV failure were present in 62 of the 110 patients on day 1. On day 2 one patient only had developed such parameters, and in 44 patients, previously noted on day 1 the parameters remained. On day 3 no further patients had developed parameters of LV failure,

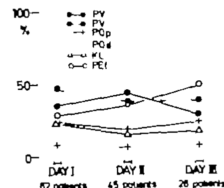


Fig. 4. Incidence of radiological signs of LV failure. Abbreviations as in Table I.

but in 28 patients previously noted on day 1 and/or day 2 the parameters remained. Thus 63 patients (57%) showed radiological evidence of LV failure.

Fig. 4 and Table I show the relative incidence of the parameters, pulmonary vessel dilatation, moderate and marked, pulmonary oedema, patchy and diffuse, Kerley's lines and pleural effusion, on days 1, 2 and 3 (62, 45 and 28 patients, respectively).

Two or more parameters were present in half of the patients with radiological evidence of LV failure. It was noted that marked pulmonary vessel dilatation, pleural effusion and Kerley's lines were present in 93%, 50% and 32% of patients, respectively, who had radiological evidence of pulmonary oedema. Pleural effusions were noted to be on the left side in 40%, bilateral in 25% and on the right side in 35%.

Ten patients had chest X-rays taken on the fourth day. All these patients had shown radiological evidence of LV failure during the first 3 days. In 6 of the 10 patients parameters indicative of failure remained. In the other 4 patients no radiological evidence of failure was present.

Standard chest X-rays taken with the patient in the standing position during the second or third week showed parameters indicative of failure in 3 of 48 patients who had shown no radiological LV failure during the first 3 days. It was interesting to note that these 3 patients had a past history of congestive cardiac failure for which they had been treated.

Parameters were absent in 48 of the 53 patients in whom parameters indicative of failure had been present during the first 3 days. In the remaining 5 patients parameters indicative of failure were noted. The parameter common to all patients with chest film evidence of failure in the second and third week was pleural effusion.

Correlation of clinical signs and radiological parameters

There are four possible categories into which the 110 patients can be placed. *Category C.* Patients with only clinical signs of LV failure. *Category R.* Patients with only radiological parameters of LV failure. *Category CR.* Patients with both clinical signs and radiological parameters of LV failure. *Category N.* Patients with no clinical signs and no radiological parameters of LV failure.

Table II. *Pattern of change of the four categories of LV failure in 70 patients during three days*

Day 1		Day 2 C	Day 2 R	Day 2 CR	Day 2 N	Day 3 C	Day 3 R	Day 3 CR	Day 3 N
C	4	2	—	—	2	—	—	—	4
R	32	—	9	11	12	2	8	2	15
CR	30	2	9	15	1	2	11	6	7
N	4	1	1	—	1	1	—	1	2

Table III. *Incidence of radiological parameters in categories R and CR*

Abbreviations as in Table I

Radiological parameter	Day 1 R (32 pats.)		Day 1 CR (30 pats.)		Day 2 R (19 pats.)		Day 2 CR (26 pats.)		Day 3 R (19 pats.)		Day 3 CR (9 pats.)	
				%		%						%
PV++	12	38	10	33	9	47	11	42	4	21	4	44
PV+++	12	38	17	57	5	26	12	46	8	42	3	33
POp	7	22	8	27	2	11	6	23	6	23	1	11
POd	1	3	4	13	0		3	12	1	5	1	11
KL	8	25	7	23	1	5	6	23	2	11	3	33
PEf	10	31	7	23	5	26	11	42	8	42	5	56

Fig. 5 shows the number of patients in each category on days 1, 2 and 3. All instances in which basal crepitations were noted were associated with radiological parameters of LV failure (in most cases patchy or diffuse pulmonary oedema). All patients in category C were noted to have a third heart sound only.

Of instances in which pulmonary oedema was reported radiologically (in the majority of cases patchy pulmonary oedema) 43% were not associated with the presence of clinical signs. The occurrence of pulmonary oedema in the absence of clinical signs was most marked on days 1 and 3 being relatively uncommon on day 2. The relative incidence of instances of radiological pulmonary oedema in the absence of clinical signs was 40%, 18% and 78% on days 1, 2 and 3 respectively.

Pattern of change of the categories

Table II shows the pattern of change of the 4 categories C, R, CR and N in patients with clinical and/or radiological parameters of LV failure.

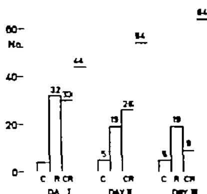


Fig. 5. No. of patients in the four categories.

The flow from day 1 to day 2 is shown in Table II and it is interesting to note that 11 patients in category R, day 1 shifted to category CR on day 2, when clinical signs developed. Thus it is evident that the bedside chest X-ray detected LV failure in 11 patients (17% of total number of patients in LV failure) one day earlier than would have been possible using clinical signs only.

It is evident that LV failure would be underdiagnosed by clinical signs alone. It is seen that 12 of the 32 patients in category R on day 1 changed to category N on day 2.

Fig. 6 and Table III show the relative incidence of radiological parameters of LV failure on days 1, 2 and 3 in categories R and CR. The relatively high incidence of patchy pulmonary oedema, which has been mentioned earlier, is readily seen on days 1 and 3 in category R.

Fig. 7 shows the incidence of LV failure on days 1, 2 and 3 if clinical signs only, radiological parameters only, or clinical signs and radiological parameters together are used to establish the diagnosis of LV failure.

Furthermore, using clinical signs only to establish the diagnosis, 83% of patients presented signs of LV failure on day 1 and 17% on day 2. Using radiological parameters only 97% presented signs of LV failure on day 1 and 3% on day 2.

DISCUSSION

As the aim of this study was to investigate the correlation between clinical and radiological signs of LV failure, a number of cases had to be excluded when clinical and/or X-ray data were incomplete. Nevertheless the incidence of LV

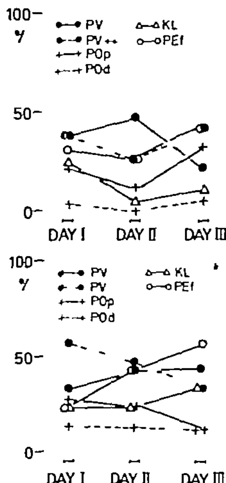


Fig. 6. Incidence of radiological parameters of LV failure within categories R (*a*) and CR (*b*). Abbreviations as in I.

failure approximates that found by some other authors (11 12, 13 14).

Basal pulmonary rales (crepitations) are a classical sign of LV failure. They result from transudation of fluid because of high pulmonary venous and capillary pressure, presumably exceeding the osmotic pressure of the plasma protein. Rales are dependent upon the presence of free fluid in the alveoli. This state of free fluid in the alveoli is known radiologically as intra alveolar oedema (10).

The other common type of pulmonary oedema is interstitial pulmonary oedema, which is unaccompanied by basal crepitations (4 10), and explains the apparent paradox, on some occasions, of the presence of pulmonary oedema radiologically in the absence of clinical signs.

The radiological parameters, patchy pulmonary oedema and diffuse pulmonary oedema, are believed to be variations of intra-alveolar oedema. A striking feature of these parameters was the rapid *change in appearance over a short period of time.* This is characteristic of intra-alveolar oedema (10).

It was interesting to note that, in the majority *of patients with radiological evidence of diffuse* pulmonary oedema, basal rales were detected. On the other hand patients with radiological evidence of pulmonary oedema, in whom basal rales were *not detected, in most instances had patchy pul*monary oedema. Of all instances in which pulmonary oedema was reported radiologically (relative incidence 40% 18% and 78% on days 1 *2 and 3, respectively) 43% were not associated* with the presence of basal rales. It is evident that intra-alveolar oedema, detectable radiologically may be present in the absence of rales. The *explanation may be that the amount of intra*alveolar fluid was insufficient to cause detectable rales, and/or the air entry into the alveoli filled with fluid was insufficient to produce rales (e.g. *hypoventilation in elderly and/or medicated pa*tients). Also the patchy type of radiological pulmonary oedema might represent a predominantly interstitial form. It is possible that rales *were present in some patients, but were missed.* However great care was taken in the determination of the presence of basal rales, and it is felt that they would not be missed in such a high *percentage of instances. Furthermore if clinical* error was the basis for the absence of basal rales

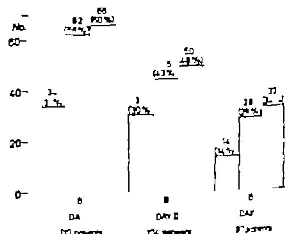


Fig. 7. Incidence of LV failure according to clinical signs only (*A*), radiological parameters only (*B*) or with both methods (*C*).

in the presence of radiological pulmonary oedema, then no variation would be expected on days 1 2 and 3 It is evident from the results that there is a significant variation from day to day probably reflecting the dynamic circulatory situation after myocardial infarction. Also, the observer's error in radiological diagnosis has to be taken into account.

Wood (18) states that in his experience *basal* rales are a misleading and often absent sign and should no longer be regarded as a sign of heart failure, as they do not occur in the absence of alveolar pulmonary oedema or bronchitis. A raised pulmonary enous pressure per se gives rise to no auscultatory signs.

It was noted that, in all patients in whom basal rales were detected, any one or more of pulmonary oedema, Kerley's lines and pleural effusion were present in the chest X-ray One possible explanation for the absence of pulmonary oedema in some of the chest X-rays was the delay of up to 4 hours between the clinical and radiological examination associated with the transient nature of intra-alveolar oedema.

The third heart sound (ventricular diastolic gallop) is a most important sign, being an early signal of LV failure (1) if other causes of a third heart sound can be excluded (e.g. constrictive pericarditis and the physiological third heart sound). It has been noted (15) that the third heart sound is almost always associated with a rise in the LV end-diastolic pressure, associated with a fall in cardiac output.

The third heart sound must be distinguished from the fourth, as it is felt that a fourth heart sound (presystolic gallop) does not have any *diagnostic or prognostic importance as a sign of* LV failure (1) and is not associated with a rise in the LV end-diastolic pressures (15) The fourth heart sound is present in the majority of patients with AMI (3 5 6) whereas the third heart sound is found less commonly In this study the third heart sound was present in 36 of patients, which corresponds to other reports (12, 16) though a figure of up to 80% has been reported (5).

The third and fourth heart sounds may overlap and give rise to a summation gallop, the significance of which is the same as, or even more serious than, that of the third heart sound (15).

A faint third heart sound may be difficult to utilize as a parameter of LV failure, as the observer error may be high. The use of phonocardiography in the CCU may possibly be of value e g. in determining the presence of a third heart sound (5 14)

Pulmonary essel (venous) dilatation appears to be the earliest radiological sign of LV failure. It has been pointed out that early in the process of LV failure the upper and lower lobe pulmonary venous tributaries are dilated to an equal degree (10) This dilatation is a direct effect of increased pulmonary venous pressure. This is an acute finding. If pulmonary hypertension is chronic, as for example in mitral stenosis or chronic LV failure then the more classical picture of upper lobe dilatation and lower lobe constriction will be seen. The prevalence of the upper lobe changes in AMI has been found to differ considerably in different series (16, 17) probably due to differences in technique. Under-perfusion of the lung bases is commonly found 6 days after myocardial infarction but then becomes less pronounced (7) The lung function may be impaired long after the resolution of the radiological changes (13)

In a study on pulmonary wedge pressure in AMI Lassers et al. (9) found a good correlation between radiographic findings of pulmonary venous dilatation or oedema, or both, and the mean pulmonary artery wedge pressure. These authors found a significant difference between the average mean artery wedge pressure in patients with normal lung fields and in those with radiological evidence of pulmonary enous dilatation or pulmonary oedema. Others, however found clinical signs more accurate in predicting a raised pulmonary artery diastolic pressure than chest X-ray (16).

Kerley's lines are due to thickening of the fibrous septa of the lung and hence are best referred to as septal lines. These lines may result from any process which thickens or infiltrates the fibrous septa. The most common cause is interstitial pulmonary oedema due to mitral valve disease, and chronic LV failure (4) Septal lines were found in a significant number of the patients in this study More than two thirds of these patients had no evidence of chronic heart failure, suggesting that septal lines are a significant parameter of acute LV failure.

Pleural effusion is a late parameter of LV failure It also occurs in right heart failure, as

the veins of the lungs and pleura drain into both the right and left atria. Wood (18) notes that left heart failure is more commonly associated with an effusion on the left side, and right heart failure with an effusion on the right side. However this appears to be controversial. Other investigators (10) have found that pleural effusion in left heart failure was more common on the right side. In this study there was no significant laterality

Another feature noted in this study was the marked transient nature of both clinical signs and the radiological parameters (particularly pulmonary oedema) It is felt that the appearance and resolution (in association with aggressive therapy for failure) of the clinical signs and radiological parameters is much more rapid in LV failure in AMI than is perhaps realized.

Thus, the earliest radiological sign of LV failure appears to be pulmonary vessel dilatation, which gives rise to no auscultatory signs, as is also the case with *interstitial pulmonary oedema* (e.g. septal lines) Furthermore, intra-alveolar pulmonary oedema detected radiologically may be present in the absence of basal rales.

It was noted that in some patients radiological parameters of LV failure were present earlier than clinical signs. It is evident therefore that chest X-rays are valuable in the diagnosis of LV failure.

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FLUOROSCOPIC SCREENING FOR LEFT VENTRICULAR ANEURYSM FOLLOWING ACUTE MYOCARDIAL INFARCTION

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Abstract. Before discharge from hospital after an acute myocardial infarction 75 consecutive survivors have been examined for postinfarction aneurysm by flouroscopy. Nine (12%) showed paradoxical pulsation of the left ventricle. Six months later the paradoxical pulsation could be observed in only five of the eight patients still alive.

It would be rational to examine patients with acute myocardial infarction (AMI) for a complicating aneurysm of the left ventricle before discharge from the hospital, and fluoroscopy might seem to be the most proper screening method considering its simplicity, harmlessness and specificity. ECG has also been used to find ventricular aneurysm (8). Persistent S-T segment elevations have been shown to occur in most patients with radiological postinfarction aneurysms (7), and patients with a rsR' pattern in V4, 5, 6 or 7 have also been demonstrated to have an aneurysm (4).

The aim of the present study was to find 1) the prevalence of aneurysms at fluoroscopy in AMI patients before discharge from the hospital, 2) the value of an ECG taken simultaneously with the screening for these aneurysms, 3) the persistence of these aneurysms 6 months later.

The criterion of an aneurysm in the present study was paradoxical pulsation of the left ventricular contour at fluoroscopy.

MATERIAL AND METHODS

The primary series consisted of 100 consecutive patients with the diagnosis of AMI. The criteria for admission, diagnosis and discharge adopted at this CCU, as well as the therapeutical policy, have been presented elsewhere (9). Twenty of the 100 patients died during the hospital stay and 5 of the survivors were not fit for fluoroscopy. The remaining 75 patients were examined by fluoroscopy for paradoxical pulsation of the left ventricular contour. The examination was performed shortly prior to discharge after 10–26 days of hospitalization. An image intensifier and television monitor system was used at fluoroscopy. The patient was in supine position on a table, where he could be rotated along his longitudinal axis. The fluoroscopic examination was performed in 7–8 positions during the 135 degrees of rotation from a right anterior oblique to a straight lateral view. In each position it was decided during maximal inspiration whether the contour of the left ventricle showed paradoxical pulsation. Locally reduced or abolished contractions did *not* give rise to the diagnosis of aneurysm.

The result of the fluoroscopic examination was related to an ECG taken at about the same time. The ECG included the leads I, II, III, VR, VL, VF, CR,R, CR, CR, CR, CR, and CR. The criterion of S-T segment elevation was 1 mm or more in the extremity leads and 2 mm or more in the praecordial leads, all in the absence of bundle branch block, ventricular strain or hypertrophy pattern.

During the 6 months following discharge from hospital 6 of the 75 patients died. Three of them were autopsied, and the autopsy reports were studied with regard to anatomical aneurysms.

All survivors with aneurysms at the fluoroscopic examination during the hospital stay were re-examined about 6 months later. At the same time another 10 patients randomly selected among the non-aneurysm cases were re-examined.

RESULTS

Nine patients showed paradoxical pulsation of the left ventricular contour at the fluoroscopic examination before discharge from the hospital. In 4 cases the location was antero-lateral, in 2 infero-lateral and in 3 inferior. In 7 of these 9 patients the localization of the infarct could be established from the ECG, and in all cases the

localization of the ECG changes and the aneurysm corresponded to each other

We were uncertain regarding the presence of paradoxical pulsation in 2 patients, who have not been included in the following presentation. In the remaining 64 patients, the control group, we were not in doubt regarding the absence of paradoxical pulsations. The 9 aneurysms correspond to a prevalence of 12% of the 75 examined AMI patients. Six were men aged 43–71 years, and 3 women aged 69–79. In the control group 43 were men aged 39–81 and 21 women aged 56–80. When remobilized before discharge from the hospital 5 of the 9 patients with an aneurysm suffered from angina on effort and 4 from dyspnoea already at rest. None had had any recorded period of ectopic tachycardia or any suspicion of cerebral embolism.

The ECG taken just before discharge from the hospital could be evaluated with regard to S-T segment elevations and rsR pattern in 7 of the 9 patients with an aneurysm and in 57 of the 64 without an aneurysm. Persistent S-T elevations were present in 29% of the aneurysm patients and in 23% among the controls. A rsR pattern in leads CR_4, CR_5 or CR_7 was found in 4% of the controls and in none of the aneurysm patients. The differences are not significant.

The chest X-ray before discharge from the hospital did not give rise to suspicion of an aneurysm in any case but revealed cardiac enlargement significantly more often in patients with an aneurysm (7/9) than in the controls (/64).

During the 6 months following the infarction 1 of the 9 patients with an aneurysm and 5 of the 64 controls died. The deceased patient with an aneurysm and 2 of the 5 controls were not autopsied. In one of the autopsied controls an aneurysm was found in an infarct, which could correspond in age to the time elapsed since hospitalization. The aneurysm was located basally in the posterior wall, and had caused death by rupturing. The fluoroscopic examination had been performed 17 days following the infarction, and the patient did not have any angina or dyspnoea, nor any S-T segment elevation or rsR pattern at that time.

The 8 survivors in the aneurysm group were re-examined by fluoroscopy 6 months following the infarction, and 5 of them still showed paradoxical pulsation of the left ventricle while the remaining 3 did not. These 3 patients had all passed their first AMI 6 months earlier while all the 3 patients with a history of previous AMI were among the 5 with paradoxical pulsation at both examinations.

Among the 10 randomly selected controls one certainly showed paradoxical pulsation, located inferiorly at this re-examination. This patient was not among the two with suspicious aneurysms at the first examination, and neither the history nor the ECG suggested another infarction in the meantime.

DISCUSSION

In this consecutive series of patients with AMI the prevalence of paradoxical pulsation of the left ventricle was 12% at a fluoroscopic examination about 2 weeks after the infarction. We have not found any fully comparable series in the literature but in one (7) 31 out of 112 consecutive patients with AMI had been examined fluoroscopically because of clinical suspicion of an aneurysm. Eighteen of them had shown paradoxical pulsation a prevalence of 16%

Fluoroscopic examination for left ventricular aneurysm is a subjective method and has in fact been shown to give both false positive and negative diagnoses with radarkymography as the reference (5). To reduce the number of false diagnoses we judged, independently of each other the ventricular pulsations in each examination position. The judgement was especially difficult in the most basal parts of the left ventricle because of the asynchronous pulsations of the adjacent atrium, and in fact we did not diagnose any aneurysm with this localization. The aneurysm diagnosed at autopsy was located basally in the posterior wall and was possibly overlooked for this reason. There may be some more false negative diagnoses in the present series for this and other reasons given by Baron (1) the aneurysm diagnosed only at re-examination could have been overlooked at the initial fluoroscopy But it was easy to recognize at re-examination and therefore one cannot preclude the possibility that it had developed after discharge from the hospital.

At the fluoroscopic re-examination 6 months following the infarction one of us was aware of the result of the primary examination Yet para-

doxical pulsation was not found in 3 of the 8 patients. None of these 3 patients had a history of a previous myocardial infarction, and the primary fluoroscopic examination occurred 11–19 days following the infarction. Besides explaining these 3 cases as false positive or negative diagnoses it is therefore possible that the paradoxical pulsation in a recent infarct will diminish as time and fibrosis go on. This possibility is supported by the observation of Dack (3) that the abnormal pulsations might disappear with time in some cases.

It may seem contradictory that some aneurysms are thought to diminish and others to develop between 2 weeks and 6 months following the infarction. This might, however be due to differences in the myocardial residue of the affected area. Lowe et al. (6) have pointed out the importance of the extension of the infarction for the formation of an aneurysm.

S-T segment elevations persisting 2–3 weeks after the infarction did not have any screening value in the present series.

To sum up in diagnosing postinfarction aneurysms a fluoroscopic examination ought to be objectivized, e.g. by kymography. Not even when defining an aneurysm as paradoxical pulsations must one however trust a negative finding. In the presence of symptoms of an aneurysm the examination must be supplemented by angiocardiography.

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COAGULATION DEFECTS AND ATHEROSCLEROSIS INDUCED IN RABBITS BY A DIET CONTAINING MEDIUM CHAIN TRIGLYCERIDES (MCT)

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Abstract. In recent years medium chain triglycerides (MCT) have been widely used in the treatment of various diseases, such as different forms of malabsorption and certain types of hyperlipaemia. Only few animal experimental studies and hardly any long-term studies had been available before the introduction of this agent as therapeutic in clinical medicine. In experiments on rabbits semisynthetic diet containing 20% MCT produced remarkable rise in serum cholesterol, certain increase of the triglycerides and distinct atheromatous changes in the aorta. The level of linoleic acid decreased markedly in the neutral lipids of serum, liver and adipose tissue. There was also an influence on the relative proportions of saturated and monounsaturated long chain fatty acids in the serum lipids. The phospholipids of serum and liver retained relatively high levels of linoleic acid. As incidental finding as that MCT caused defect of the coagulation of the same type as that seen in severe diseases of the liver, such as liver cirrhosis in man. At postmortem examination considerable changes were found in the liver, probably manifestation of toxic effect. In two cases MCT was replaced by an equivalent amount of corn oil after 32 eeks. This resulted in rapid normalization of the serum cholesterol and triglyceride values. The coagulation of the blood also became normal. Postmortem examination of these two cases, after they had been fed corn oil for 18 weeks, revealed normal or practically normal liver.

Earlier investigations on the ffect of various fatty acids on serum cholesterol

Ever since 1952, when Kinsell et al. (27) and Groen et al. (20) simultaneously showed that a diet containing vegetable fat reduced the blood cholesterol while fat of animal origin raised th cholesterol, much interest has been focused on the significance of fat in the development of atherosclerosis. Detailed investigations (2, 6, 12, 34 and others) have shown that the effect of a given fat on the blood cholesterol depends on its chemical composition rather than on its origin. These investigations showed that fat containing mainly saturated fatty acids had an enhancing effect on serum cholesterol fats rich in polyunsaturated fatty acids had a depressive effect. It has also been shown in experiments on rabbits fed a semisynthetic diet containing triglycerides of different fatty acid composition that lauric acid (C12) and myristic acid (C14) have the strongest enhancing effect on serum cholesterol. Capric acid (C10) caprylic acid (C8) and also palmitic acid (C16) have a weaker effect (35 36, 37).

Coconut fat contains 50% C12 and approximately 20% C14 which may explain the strong cholesterol enhancing effect of this egetable fat. In similar experiments on rabbits it was shown that the depressive effect of liquid egetable oils on serum cholesterol is due to the abundance of linoleic acid (C18 2) in such fat.

Medium chain triglycerides

The physiological effect of various saturated f tty acids, the pathways of their resorption and their effect on the cholesterol turnover received increasing attention after the chemical industry had succeeded in producing a semisynthetic fat containing mainly triglycerides of C8 and C10. This fat was called medium chain triglycerides (MCT) It was obtained as a byproduct on fractionation of coconut fat.

The results of investigations on the effect of

MCT on serum cholesterol in animals have varied widely Kaunitz et al. (26) used rats, which were given an MCT preparation for 3 months. It contained 6- to 12-carbon triglycerides but no C18. The serum cholesterol levels were relatively low (61 mg/100 ml). The control animals, which were given a corresponding amount of lard, had somewhat higher values (83 mg/100 ml)

Kritchevsky and Tepper (28) reported the following levels of total cholesterol in serum (mg/100 ml) of rats fed a diet containing 20% fat: 54 on MCT 77 on coconut oil, 85 on corn oil and 66 in control animals on rat chow They used only 5 rats in each experimental group. Some of the other experiments performed simultaneously are difficult to interpret because the diet contained also 2% cholesterol and 0.5% cholic acid.

Fischer and Kaunitz (15) compared the response of chickens and of rats given either MCT or LCT (produced from coconut fat and containing C12–C18) The plasma cholesterol was 162 mg/100 ml in chickens fed 12% MCT and 141 on the LCT diet. In rats, on the other hand the cholesterol values were somewhat lower in the animals fed MCT than in those fed LCT (68 and 96 mg/100 ml, respectively)

Whiteside et al. (46) found the following serum cholesterol values in chickens fed a diet containing 15% different fats for 4 weeks. 178 mg/100 ml when the diet contained coconut fat, 152 on MCT and 127 on a corn oil diet Grande (19) ve dogs a diet containing C12 and C14 and then d the cholesterol values to be higher than on a diet containing C16 and C18 or C8 and C10 The MCT diet (C8 and C10) produced only slightly higher cholesterol levels than a low fat diet with 4% fat calories.

Kritchevsky and Tepper (29) carried out some complicated experiments on rabbits. They used a diet containing 2% cholesterol and different sorts of fat. Coconut fat proved more atherogenic than corn oil. MCT and tristearin were equally atherogenic as corn oil, but serum cholesterol was somewhat lower As far as we know no experiments have been performed on rabbits with MCT without supplementary cholesterol.

Though these animal experiments are thus fairly scanty and have not given conclusive results, MCT has been widely used in clinical trials and therapeutically The first clinical trial with MCT was published by Blomstrand et al. (9) A 52 year-old woman with chyluria was given a test meal containing 20 g MCT No increase in urinary lipid resulted and no MCT could be detected in the urine. It was therefore concluded that the short chain fatty acids were not absorbed via the lymphatics. This agrees with the earlier animal experiments by Bloom et al. (10) In trials with ^{14}C-labelled fatty acids of different chain lengths in rats with a cannula inserted in the thoracic duct, these authors found that 15–55% of the lauric acid and 5–19% of the capric acid could be recovered in the lymph. The major portion of the short chain fatty acids were transported via the blood stream from its site of absorption. Borgström (11) has shown in similar experiments on rats that the largest part of C10 is absorbed via the portal vein as the free acid. In this respect they differ strikingly from the long chain fatty acids in the lymph, which are transported exclusively in the ester form. In analogous experiments Blomstrand (8) recovered 3–16% of C10-1-^{14}C in the lymph lipids. Phospholipids contained 2% of the activity and the remainder was found in the neutral fat. Hashim (22) who studied dogs, found an extensive hydrolysis of the medium chain triglycerides in the intestine and subsequent transport of the split fatty acids (as free fatty acids) in the portal vein. The medium chain fatty acids reaching the liver did not subsequently appear in significant quantities as free fatty acid esters in hepatic venous blood but as water-soluble products.

Therapeutic use of medium chain triglycerides

Various experiments on healthy persons and patients with different malabsorption diseases have shown that medium chain triglycerides are rapidly hydrolyzed in the intestine and that their fatty acids are rapidly absorbed by intestinal epithelial cells into the portal vein and transported to the liver This holds also for patients with impaired absorption of ordinary fat containing long chain fatty acids. MCT has therefore been widely used in the treatment of steatorrhoea associated with a variety of malabsorptive disorders, such as cystic fibrosis of the pancreas, abnormal lymphatic drainage of the intestine in intestinal lymphangiectasis and resection of large portions of the small intestine. In several cases treatment with MCT diet proved successful, steatorrhoea dimin-

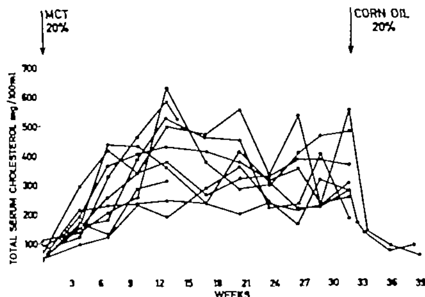


Fig. 1. Total serum cholesterol in rabbits fed on a semi-synthetic diet containing 20% MCT. In two rabbits MCT as replaced by corn oil after 3? weeks.

lished, body weight increased, and the patient's general condition improved.

MCT has also been used in the treatment of β-lipoprotein deficiency. Isselbacher et al. (24) and Fredrickson et al. (17) have suggested that the defect in this congenital disease should be sought in the removal of dietary long chain triglycerides into the lymph, probably as a result of failure to form chylomicrons. Isselbacher et al. demonstrated that such patients could utilize MCT which is transported to the liver via the portal vein. The treatment with MCT resulted in weight gain and diminution of intestinal symptoms. A fatty liver was, however found in one of the patients treated with MCT (24). Law (31) has also reported fatty liver in a case of a-β lipoproteinaemia treated with MCT.

The effect of MCT on serum lipids in humans has been the subject of several investigations. Beveridge et al. (7) gave 30 cal.% as MCT in a formula diet for 8 days to a group of healthy university students. They observed no significant change in serum cholesterol levels when a fat free regimen was replaced by the MCT formula. Hashim et al. (23) gave a formula diet containing 40 cal.% of different kinds of fats to eight patients. On an MCT formula they observed only an insignificant increase of serum cholesterol —about 10% higher than with a corn oil formula. Serum cholesterol increased on butter after MCT and fell when butter was replaced by MCT. Uzawa et al. (45) who tested the effect of MCT on three patients including two diabetics, found an increase of serum triglycerides when the patients were on an MCT diet, which, however disappeared after replacement of the MCT by saf flower oil. Kuo and Huang (30) also found MCT to produce a certain elevation of serum triglycerides in infants with cystic fibrosis of the pancreas. Ahrens and Spritz (3) used MCT in the treatment of fat-induced hyperglyceridaemia. It is believed that the lipaemia in such subjects is due to a prolonged retention of chylomicrons in the blood stream because of a defective lipolysis. When MCT is the sole source of dietary fat, C8 and C10 are mainly transported in the form of free fatty acids via the portal vein to the liver. Only a small fraction of C10 is transported in the ester form via the lymphatic route. Consequently the hyperchylomicronaemia subsides. The fatty acid composition of plasma triglycerides was similar to that on carbohydrate feeding. Furman et al. (18) also proved the MCT treatment of fat induced hyperglyceridaemia to have a good effect.

Our animal investigations

It is clear from the review of the literature that previous animal experiments have not convinc

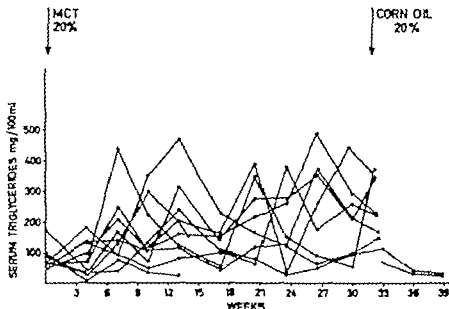


Fig 2 Serum triglycerides in the same rabbits as in Fig. 1

ingly shown whether MCT has any effect on the serum lipids. Neither have any comprehensive long-term experiments been carried out on suitable animals to discover whether saturated fatty acids with relatively short carbon chains (C8 and C10)—*in contrast to C12 or C14*—lack any atherogenic effect. It is naturally also of interest to ascertain in long-term experiments—*not only on rats but also on other experimental animals*—ether MCT has any serious side-effects.

MATERIAL AND METHODS

W ha used male rabbits of Swedish native breed in series of experiments lasting for 32 weeks when the animals were given well-defined, semisynthetic diet in the form of pellets. The diet contained (by wt.) 20% MCT and no other fat, 20% beet starch, 8% dextrose, 25% casein, 17% cellulose and adequate amounts of vitamins and salt. Water was allowed ad lib. The animals, which ere kept in separate hutches, had good appetite. The average increase in weight was 640 g during the experimental period of 32 weeks. The MCT was obtained from AB Karlshamns Oljefabriker Karlshamn, Sweden. It had the following fatty acid composition (wt.%):

6 0	8 0	9 0	10 0	12 0	14 0	16 0	18 0	18 1
1.9	63.9	0.2	29.9	1.3	0.2	0.6	0.4	0.9

18 2	18 3	20 0	22 1
1.0	0.3	trace	0.6

Blood samples for determination of cholesterol and analysis of triglycerides were obtained from the ear after 12 14 h fasting. As a rule these samples were obtained every third week throughout the experimental period. The total serum cholesterol was determined by the method of Abell et al. (1) as modified by Anderson and Keys (4). The method was continuously checked by analysis of unknown samples from the Lipid Standardization Laboratory Atlanta, USA. The glyceride-glycerol content of the serum as measured by the method of Carlson and Wadström (13).

In a pilot experiment on three rabbits the serum cholesterol rose considerably and attained the following values after 15 weeks: 185 352 and 525 mg/100 ml. A new lot of MCT was obtained from the oil mill and new series of experiments was started on ten rabbits. Also in this experiment, which was continued for 32 weeks, the serum cholesterol showed a clear rise (Fig. 1). The serum triglycerides increased, but not quite as much as the cholesterol (Fig. 2). In two cases the diet was changed after 32 weeks. The MCT was replaced by 20% corn oil, hich caused considerable fall of both cholesterol and triglycerides in serum, both of which reached normal levels after some weeks.

Fatty acid analysis of serum, liver and adipose tissue lipids

The lipids wer extracted with chloroform-methanol (2/1, /) and fractionated by thin layer chromatography into cholesterol esters, triglycerides, free fatty acids and phospholipids. The fatty acid composition of the isolated fractions was determined by gas-liquid chromatography. Details of the analytical procedures are described elsewhere (3).

RESULTS

The fatty acid composition of serum, liver and adipose tissue lipids is given in Table I. In each

Table I. *Fatty acid composition of serum, liver and adipose tissue lipids*

The fatty acids are designated by (number of carbon atoms) (number of double bonds). The figures are individual values except for the serum values of normally fed rabbits, where they are means ± S.D. for three animals

Lipid fraction	Fatty acids (wt. %) 10 0	12 0	14 0	16 0	16 1	18 0	18 1	18 2	20 4
Serum									
Cholesterol esters									
Normally fed	—	—	0.5 ± 0.2	16.9 ± 0.9	4.6 ± 1.1	3.3 ± 0.5	31.1 ± 8.5	43.5 ± 8.4	—
MCT 14 weeks	—	—	1.3	19.7	11.0	3.7	50.3	14.1	—
MCT 22 weeks	—	—	0.9	18.5	5.1	19.4	38.0	18.2	—
MCT 32 weeks	—	—	1.3	16.5	6.8	8.1	46.0	21.2	—
MCT 32 weeks then corn oil 18 weeks	—	—	0.1	10.3	0.6	1.8	25.2	62.0	—
Triglycerides									
Normally fed	—	—	2.2 ± 1.1	30.8 ± 6.7	5.5 ± 0.8	4.7 ± 1.3	36.3 ± 7.1	20.5 ± 3.8	—
MCT 14 weeks	—	1.9	3.8	40.3	8.3	4.9	35.6	5.1	—
MCT 22 weeks	—	Trace	2.7	41.7	3.2	15.3	28.0	9.2	—
MCT 32 weeks	—	Trace	4.2	35.2	15.5	10.4	22.7	12.0	—
MCT 32 weeks then corn oil 18 weeks	—	—	0.3	18.4	0.8	4.2	24.6	51.8	—
Free fatty acids									
Normally fed	—	—	3.5 ± 1.1	34.5 ± 2.8	5.8 ± 0.5	11.7 ± 4.6	22.8 ± 4.2	21.8 ± 2.9	—
MCT 14 weeks	—	Trace	14.5	39.2	2.6	8.0	29.3	6.3	—
MCT 22 weeks	—	Trace	7.5	44.4	2.9	19.5	19.5	6.3	—
MCT 32 weeks	—	5.4	5.8	34.0	7.3	10.1	22.1	13.1	—
MCT 32 weeks then corn oil 18 weeks	—	0.3	0.9	23.6	1.1	9.8	23.1	41.3	—
Phospholipids									
Normally fed	—	—	0.2 ± 0.2	24.2 ± 3.2	0.9 ± 0.7	21.5 ± 4.0	14.0 ± 3.9	33.1 ± 3.3	6.2 ± 1.5
MCT 14 weeks	—	—	0.4	27.4	2.4	17.9	29.1	20.3	2.5
MCT 22 weeks	—	—	0.2	21.0	1.0	25.0	17.2	30.0	5.8
MCT 32 weeks	—	—	0.2	20.0	1.4	23.2	17.9	33.6	3.7
MCT 32 weeks then corn oil 18 weeks	—	—	—	18.1	—	23.6	9.2	49.0	—
Liver									
Cholesterol esters									
Normally fed	—	—	0.2	35.6	4.5	9.1	32.7	17.7	—
MCT 32 weeks	—	—	3.4	18.3	8.0	10.3	52.2	7.7	—
MCT 32 weeks then corn oil 18 weeks	—	—	—	8.3	0.6	4.1	41.8	45.3	—
Triglycerides									
Normally fed	—	—	1.9	40.0	5.7	3.0	28.2	21.2	—
MCT 32 weeks	—	Trace	5.2	46.1	5.5	9.0	26.9	7.2	—
MCT 32 weeks then corn oil 18 weeks	—	—	0.6	21.6	1.0	3.5	21.6	51.6	—
Phospholipids									
Normally fed	—	—	—	15.9	0.5	27.6	11.4	35.6	8.0
MCT 32 weeks	—	—	0.2	16.6	2.1	24.0	21.1	29.3	5.7
MCT 32 weeks then corn oil 18 weeks	—	—	—	13.6	0.2	26.4	7.8	44.0	6.0
Intraperitoneal adipose tissue									
Triglycerides									
Normally fed	—	—	2.8	32.9	5.8	6.7	30.7	21.1	—
MCT 32 weeks	3.7	1.4	7.5	36.0	7.5	4.7	30.6	8.6	—
MCT 32 weeks*	3.7	1.3	7.1	36.1	6.5	6.4	30.0	8.8	—
MCT 32 weeks then corn oil 18 weeks	—	—	0.9	13.5	1.0	3.6	28.0	53.0	—

* Subcutaneous.

Table II. *Coagulation data*

	After 22 weeks MCT diet Rabbit no.				After 32 weeks' MCT diet Rabbit no.						After 18 weeks' corn oil diet Rabbit no.		
	1	2	3	Normal	2	4	5	6	7	Normal	5	7	Normal
Recalcification time (sec)	122	175	116	72	210	140	169	160	150	108	112	85	150
Partial thromboplastin time (sec)	154	168	132	92	172	130	142	190	161	106	132	130	138
One-stage prothrombin time (sec)	17	18	18	15	15	14	14	16	19	13	20	20	20
P&P (%)	50	63	45	100	60	59	42	51	37	100	86	93	100
Factor V („)	83	68	70	100	70	70	64	56	63	100	86	85	100
Fibrinogen (g/100 ml)	0.12	0.14	0.14	0.29	0.21	0.26	0.13	0.10	0.10	0.29	0.37	0.25	0.27
Thrombin time (sec) (3 NIH U/ml)	24	36	24	20	23	21	20	38	39	23	30	28	30
Fibrinolytic activity (mm^2) (fibrin plates)													
Plasma	0	0	0	0	0	0	0	0	0	0	0	0	0
Resusp. euglob. prec.	34	33	39	0	0	0	0	0	40	0	0	0	0
Euglobulin clot lysis time (min)	20	20	35	>240	240	240	240	35	35	>240	>240	>240	>240
Urokinase inhibitors (%)	55	56	72	100	91	85	71	92	72	100	108	117	100
Platelet count/mm^3				400 000		148 000		168 000	46 000	465 000			

fraction the listed fatty acids represented approximately 90% of the fatty acids with more than 10 carbon atoms. The remaining 10% were accounted for by several minor components which were not identified.

The relative proportions of saturated and mono- fatty acids in the serum lipids varied considerably during the feeding of MCT while the concentration of C18 decreased markedly in both serum, liver and adipose tissue, with one exception. In the phospholipids of serum and liver C18. 2 was retained at quite high levels. It therefore seems unlikely that the function of phospholipids as essential components of vital lipoprotein structures would have been severely impaired in the present experiments.

The incorporation of C10 in adipose tissue was quite low after 32 weeks of MCT feeding. This may be related to the very low level of C18 2 (1%) in the diet, since Kaunitz (25) found that incorporation of MCT into tissue triglycerides occurred only when the diet contained relatively high levels of C18 2.

The changes seen in the fatty acid patterns during the feeding of MCT were completely abolished by 20 weeks of corn oil.

COAGULATION STUDIES

When the experiment with MCT had been in progress for 3 months it was observed that blood samples did not coagulate in normal way in the centrifuge tube. Clot formation was severely retarded and the clot was so loose that only little serum was obtained when the sample had been allowed to stand. In order to find an explanation for this disorder the following examinations were carried out at the Coagulation Laboratory Malmö General Hospital.

Blood samples were obtained by heart puncture. About 15 ml blood was taken from each rabbit. Serum was prepared from blood collected in glass tubes. For preparation of citrated plasma 8 ml blood was collected in siliconized glass tubes containing 2 ml 3.8% trisodium citrate solution.

The following determinations were made: one-stage prothrombin time, partial thromboplastin time, recalcification time, the prothrombin group—P&P (factors II, VII and X), factor V fibrinogen, Thrombin time, euglobulin clot lysis time, fibrinolytic activity of plasma and resuspended euglobulin precipitate on unheated fibrin plates and urokinase inhibitors. Methods described previously were used (39 40, 41 42, 43).

Pooled plasma and serum from five normal rabbits were used as standard. (The blood was collected at the same time as the rabbits to be investigated.)

RESULTS

The results of the coagulation and fibrinolytic studies are summarized in Table II. After 22 and

32 weeks MCT diet all the investigated rabbits showed coagulation defects. The platelet count showed a decrease. The recalcification time and the partial thromboplastin time were prolonged. All had decreased values for P&P and factor V The fibrinogen content was decreased in six rabbits. In five rabbits the euglobulin clot lysis time was considerably shorter than in the control animals, and in four of these rabbits fibrinolytic activity could also be demonstrated on fibrin plates. In normal rabbits it is never possible to demonstrate any fibrinolytic activity on fibrin plates. The thrombin time was prolonged in all but three rabbits. The urokinase inhibitors showed a small decrease. In the two animals, which were tested after replacement of the MCT diet by corn oil for 18 weeks, the changes in the coagulation and fibrinolytic system had returned to normal.

DISCUSSION OF THE COAGULATION DEFECT

After feeding the rabbits with MCT diet for 22 and 32 weeks a coagulation defect developed, characterized by decreased values for the prothrombin group factor V and fibrinogen and in most animals also by increased fibrinolytic activity The thrombin time was prolonged and the urokinase inhibitors somewhat decreased. In addition, the platelet count tended to be lower than in the control animals. These changes in the coagulation and fibrinolytic pattern are similar to those seen in liver cirrhosis in man (14 16 21). Apart from a defectiv synthesis in the liver of the prothrombin group, factor V and fibrinogen and a defective clearance of fibrinolytic activators of the liver the coagulation changes may also to some extent be due to intravascular coagulation induced by the lipid diet. On pathological examination of the various organs no signs of generalized intravascular coagulation with microthrombi were seen but, as pointed out below significant liver damage. This suggests that the main reason for the coagulation defect was impaired liver function.

MORPHOLOGICAL CHANGES

Since much blood was needed for the coagulation studies heart puncture had to be performed. One animal died

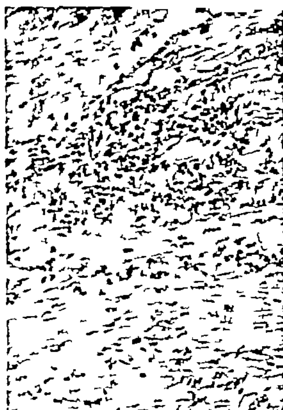


Fig 3 Inflammatory focus in the myocardium (Rabbit 421 – no. 4 in this series. HTX-eosin, 160)

within 1 hour after heart puncture. The postmortem showed haemorrhage into the pericardial sac but no other haemorrhages or macroscopical changes. Microscopically lungs, liver, spleen, kidneys, suprarenals and testes were normal. The myocardium showed small areas of interstitial fibrosis and some medium-sized intramural arteries showed intimal hyperplasia, occasionally with few foam cells and with slight reduction of the lumen. In aorta minimal lipid streaks were found in relation to branches.

Another animal died spontaneously after 18 eeks, and the postmortem did not reveal the cause of death Microscopically heart, lungs, liver suprarenals and kidneys were normal, testes were atrophic. Aorta showed few minimal lipid streaks in relation to branches.

Of the remaining seven rabbits four were killed after 32 weeks, and fifth died spontaneously two days after heart puncture performed hen the above mentioned animals were killed. In these five rabbits lungs, spleen, kidneys and testes were normal microscopically In one animal the suprarenals showed infiltration of inflammatory cells but no necroses. The heart showed changes in all five animals. In one rabbit only minimal interstitial calcifications were found, but the remaining four showed small areas infiltrated with inflammatory cells but hout any muscle necroses (Fig 3). I one of these animals the



Fig 8. Aorta, coloured with Sudan. Note the extensive lesions covering most of the thoracic aorta. (Rabbit 425 – no 2.)

cytes. Many of them contained needle-shaped vacuoles, probably after dissolved cholesterol crystals, even though they did not show any birefringence. Cholesterol crystals were, however found in other organs.

COMMENT

A search of the available literature revealed no report of coagulation disorders ascribable to MCT used in animal experiments, in trials on healthy human volunteers or in treatment of various human diseases. Obviously this is why laboratory studies of MCT for its effect on coagulation have been considered unnecessary Leyland et al. (32) used MCT in the treatment of 13 children with malabsorption syndrome. One of the children had neutropenia and thrombocytopenia and died from pulmonary haemorrhage after half a year's treatment with MCT The haematological symptoms had, however started before the treatment with MCT Yssing et al. (47) described a case of protein-losing enteropathy treated with skimmed human milk supplemented with MCT The patient was suspected of being hypersensitive to cow's milk. When, on one occasion, human milk was replaced by a milk formula made of cow's milk, the child started vomiting and a few hours later passed watery stools containing fresh blood. It is, however less likely that this bleeding had anything to do with MCT therapy but was rather a manifestation of allergy to cow's milk protein.

We have not yet been able to study the coagulation status in patients treated with MCT The experiments in rabbits do not, of course, warrant any conclusions concerning the effect of MCT on coagulation in humans. But since an effect of MCT cannot be excluded, it might be justified to watch the coagulation status in patients receiving MCT and particularly in patients with severe malabsorption in whom bleedings also may appear due to vitamin K deficiency

In the rabbits fed a high MCT diet the coagulation defect was most likely due to liver injury Considerable liver changes were also found at post-mortem examination. The underlying mechanism of such changes is still not properly understood. Various possibilities might be considered, such as a direct toxic effect of C8 and C10 which in the form of free fatty acids are transported in large amounts via the vena porta to the liver after every meal. If the enzymatic system that is to break down this fat cannot cope with such postprandial loads, the free fatty acids may have an injurious effect on the liver cells. We found no signs of a fatty liver at pathological examination of our rabbits fed MCT A fatty liver has, however been described by Isselbacher et al. (24) and Law (31) in patients with β-lipoprotein deficiency treated with MCT

Previous findings in rabbits have taught us that these animals often develop liver damage, not infrequently leading to liver cirrhosis when fed a diet which is inadequate in any respect. Examples of such experiments follow: commercial pellets containing 1% cholesterol, semisynthetic diet with 8% hydrogenated coconut oil, but without supplementary cholesterol, the same semisynthetic diet with added glyceryl trilaurate (C12) semisynthetic diet with 50% saccharose but without fat or cholesterol. Common to all of these experimental diets was that they affected the liver in such a way that they sometimes resulted in liver cirrhosis. In these earlier experiments we did not notice any signs of impaired coagulation

status of the animals. But we did find clear hypercholesterolaemia, and as a rule it was so severe as to cause widespread atherosclerosis within a year. That our semisynthetic diet as such did not contain any injurious factor is apparent from the fact that the same diet, but with added oil rich in C18 2, such as corn oil, instead of saturated fat, did not cause hypercholesterolaemia, atherosclerosis or liver injury. In an earlier experiment (37) we showed that it is possible to compensate for the atherogenic and hepatogenic effect of 4% glyceryl trilaurate by addition of 6% linoleate. Also in the present experiment the cholesterol level and the triglyceride values became normal on replacement of MCT in the diet by an equivalent amount of corn oil. The coagulation likewise became normal. It was also found that at the end of the experiment, about 18 weeks after the change of diet, postmortem showed the liver to be of largely normal appearance.

Our experiments are incomplete insofar as we did not ascertain whether addition of a certain amount of polyunsaturated fat would counteract the effect of MCT on the coagulation system.

Our knowledge of the atherogenic effect of MCT is also still scanty. It has been generally expected that MCT, in contrast to other saturated fat, would have no such effect, at least not in humans, since MCT does not produce any appreciable increase of the serum cholesterol. This is probably because MCT after absorption is transported direct to the liver where it is broken down. It has been postulated that the metabolism of MCT resembles that of carbohydrates more than that of any other saturated fat. Why then, as shown in our experiments, does MCT cause such an increase of the serum cholesterol in rabbits resulting in a clear though not severe atheromatosis? One explanation might be that, in rabbits more than in other animals and humans, MCT is absorbed via the lymphatic and that the fat is consequently presented to the blood stream without first passing through the liver. To clarify this question it would be necessary to study the metabolism of MCT in rabbits after cannulation of the thoracic duct, e.g. by the technique described by Zilversmit et al. (48). We have not had the oportunity to carry out such experiments.

Theoretically one might also imagine another explanation of the hypercholesterolaemia and atheromatosis occurring in rabbits fed a high MCT diet. If free C8 and C10 supplied to the liver in the portal circulation produce any injury of the liver it might result in a disorder of the lipoprotein synthesis, which might in turn cause atheromatosis. Linscheer's observations (33) in cirrhotic patients treated with MCT may perhaps be of interest in this connection. He found a high concentration of free C8 and C10 in the systemic circulation following oral administration of MCT. After surgical portacaval shunt the amount of FFA increased considerably. Schwabe et al. (44) demonstrated toxic reactions after i.v. administration of 2 g sodium octanoate. Muto et al. (38) discussed the possibility of a diffusion of free C8 and C10 across the blood–brain barrier in patients with hepatic coma.

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SPLENECTOMY — INDICATIONS AND RESULTS

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Abstract. 182 splenectomies performed in two large hospitals during a 20-year period have been analysed. The frequency of splenectomies has risen, especially during the last 5-year period. "Surgical" indications are predominantly traumatic lesions of the spleen. The most common "medical" indications have been haemolytic anaemia and thrombocytopenia. No death has occurred ascribable to the splenectomy as such. It is emphasized that splenectomy in many instances should be regarded as one of several therapeutic possibilities and that it should not be saved as "last resort.

The interest in splenectomy as a therapeutic measure has risen during the last few years. Nevertheless, our knowledge of what happens when the spleen is removed is insufficient, both when it is done because of trauma and perhaps even more when splenectomy is done as a therapeutic procedure in disease. Much basic research is needed on the normal and pathological physiology of the spleen and its implication for circulation, hematopoiesis and immune response, as well as on the clinical course of splenectomized patients.

In order to try and form a policy regarding splenectomy it was found necessary to analyse the hitherto prevailing reasons for the operation. This study is based on a survey in the splenectomies performed during the last 20 years in two major Stockholm hospitals.

MATERIAL

All patient records indicating splenectomy have been scrutinized for the period 1950–69 from two large Stockholm hospitals, the Serafimer Hospital in Stockholm City one of the University Hospitals of the Karolinska Institute, and the Danderyd Hospital, central hospital for the County of Stockholm.

Indications for splenectomy may in some patients be of "medical" as well as "surgical" nature, e.g. in hepatic cirrhosis. The division made in this study is entirely dependent upon the department in which the decision to perform splenectomy was originally taken.

RESULTS

Altogether 182 patient records were available for the study

The number of splenectomy patients in the different departments and hospitals is shown in Table I. The number of splenectomies has been constant during the years 1950–64 but rose during the last 5-year period—in both hospitals—to 3–4 times the previous level. The figures relative to the number of admissions (Table I B) however show that a great part of this change is due to an increase in admissions. Only in the Department of Surgery at the Serafimer Hospital was there a marked relative increase of splenectomies during the last 5-year period.

The age distribution is shown in Fig. 1. It is evident that there exist two maxima, one around 20 years of age, the other around 50. The composition of these maxima with regard to diagnoses is given in Table II.

There are slightly more females (99 = 54 %) than males (83 = 46 %) in the total material. This difference is not statistically significant. If the material is divided into medical and surgical patients there is, however a preponderance of women in the medical (69 %) as compared to the surgical (43 %) material, the difference now being statistically significant (χ^2-test, $p < 0.01$). Even if the traumatic ruptures, of which the majority occurred in males (74 %), are excluded, there still remains a predominance of females in the medical material. As seen from Table III, this is due to

Table I. *Frequency of splenectomy in two large hospitals*

Time	Serafimer Hospital Medical	Serafimer Hospital Surgical	Danderyd Hospital Medical	Danderyd Hospital Surgical	Both Medical	Both Surgical	Total
A. *Absolute figures* (No. of splenectomies during a 5-year period)							
1950–54	11	1	7	7	18	8	26
1955–59	14	6	7	4	21	10	31
1960–64	7	10	5	8	12	18	30
1965–69	15	49*	15	16	30	65	95
	47	66	34	35	81	101	182
	113		69		182		
B. *Relative figures* (No. of splenectomies/1000 admissions and year)							
1950–54	1.0	0.1	0.6	0.3			
1955–59	1.3	0.4	0.6	0.2			
1960–64	0.7	0.7	0.4	0.4			
1965–69	0.9	3.2	0.9	0.7			
Whole period	1.0	1.0	0.6	0.4			

For comments see text. Includes 9 splenectomies in connection with bilateral nephrectomy and 9 in conjunction with total gastrectomy for gastric carcinoma performed during 1966–67

the high proportion of females among the non-hereditary forms of haemolytic anaemias and the thrombocytopenias, two conditions more common in females than in males.

The diagnoses and indications for splenectomy separated into medical and surgical are given in Tables III–IV.

The traumatic ruptures are separately analysed in Table V.

Postoperative complications are listed in Table VI. The results of a limited follow-up are given in Table VII.

Table II. *Age influence on the indications for splenectomy*

	Younger group (mean age 20)		Older group (mean age 50)	
Medical indications				
Haemolytic anaemia	8		14	
Hereditary spherocytosis	5		3	
Other forms	3		11	
Thrombocytopenia	8		6	
Hepatic cirrhosis	7		2	
Malignant lymphoma	—		5	
Miscellaneous	5		7	
	28		34	
Surgical indications				
Traumatic rupture	26	84 %	6	
Malignant neoplasm	—		13	38 %
Unintentional damage	—		9	26 %
Miscellaneous	5		6	
	31		34	

DISCUSSION

The first large series of splenectomies was collected by Mayo (15) who in 1928 reported the results of 500 splenectomies. He pointed out that good—and sometimes prolonged—remissions could be obtained from splenectomy in leukaemia and lymphomas. Many reviews and studies have been published since. From later years, those of Sandusky et al. (20) Lowdon et al. (13) Nordøy and Neset (17) Gomes et al. (10) and Egelund et al. (5) may be mentioned. They all discuss indications in haematological diseases. Sandusky et al. state that with the exception of the well established indications, hereditary spherocytosis and idiopathic thrombocytopenic purpura, the outcome in other haematological diseases was, at the best, "problematical" and included a surgical mortality of almost 9%. Nordøy and Neset are more optimistic in their conclusions from 179 patients and emphasize that more than 50% of patients with acquired haemolytic anaemia benefited from the operation. Egelund et al. point, among other facts, to the high frequency of postoperative complications in patients with chronic lymphatic leukaemia. Lowdon et al. analysed the reasons for splenectomy in a large British material (1 100 patients) and mentioned the risk of serious infections following splenectomy. They concluded that splenectomy should be avoided during the first four years of life but that otherwise no authoritative estimate could be made regarding the risk of postsplenectomy infections. Other authors state that splenectomy should be avoided before 2 years of age (10)—although Broberger et al. (3) found no signs of increased susceptibility to infections in children who had been splenectomized, nor did others in adults (1, 6, 18). All authors seem to have one thing in common, viz. the relative uncertainty of what the real value—and the risk—of splenectomy is.

Already many years ago it was stated that the interest in splenectomy had increased (7). Our

Table III. *Medical indications for splenectomy*

	Men	Women	Total
Haemolytic anaemia			
Hereditary spherocytosis	4	6	
Other forms	4	10	24
Thrombocytopenia essential	3	14	17
Hepatic cirrhosis			
With thrombocytopenia	2	5	
With portal hypertension	1	1	9
"Hypersplenism	1	8	9
Malignant lymphoma	4	4	8
Felty's syndrome	—	3	3
Myeloid leukaemia	—	2	2
Gaucher's disease	1	1	2
"Pseudoleukaemia	1	1	2
Aplastic anaemia	—	1	1
Phaeochromocytoma	1	—	1
Infectious mononucleosis (ruptured spleen)	1	—	1
"Thorotrast spleen	1	—	1
Fever of unknown origin — reticulosis	1	—	1
	25	56	81

Splenic rupture during splenoportography

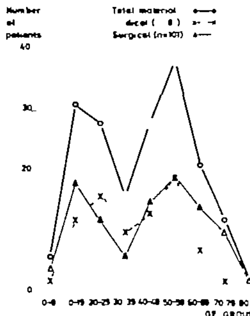


Fig. 1. Age distribution of splenectomized patients. – medical indications, Δ—Δ – surgical indications, O—O – total material.

figures show that the absolute frequency of splenectomy has been constant during a 15-year period, but during the last 5 years has gone up.

Table IV. *Surgical indications for splenectomy*

	Men	Women	Total (*n*)	(%)
Traumatic rupture	30	11	41	41
Splenectomy in cancer surgery				
Carcinoma of the stomach	12	5		
Carcinoma of the large bowel	—	2		
Carcinoma of the kidney	—	1		
Carcinoma of the pancreas	2	2		
Intraperitoneal metastases	—	1		
			25	25
Splenic damage during other operations				
Stomach surgery				
Carcinoma	1	2		
Ulcer	3	7		
Hiatus hernia	1	3		
Gall bladder surgery	—	1	18	18
Bilateral nephrectomy	5	6	11	
Pancreatic surgery	2	1	3	
Hepatic cirrhosis shunt operation	1	—	—	
Reticular cell sarcoma	—	1	1	
Fistula of the left colon	1	—	1	
	58	43	101	

T additional ruptures are listed in Table III medical indications.

It may also be noted that the frequency during the entire 20-year period has been approximately 40–50% higher in the University Hospital, absolute as well as relative, than in the County Hospital. Especially high figures were recorded in the years 1966–67 due to trials with radical gastrectomy for gastric carcinoma and splenectomies performed in connection with bilateral nephrectomy before renal transplantation.

The age distribution curve has two maxima, for the entire material as well as for the medical and surgical groups (Fig. 1). The medical material has roughly the same composition in both age groups, mean age 20 and 50 years, respectively, whereas the surgical material is entirely different in the two groups. The younger group is dominated by the traumatic ruptures (84%) with a strong male predominance. In the older group there are only a few ruptures, some of unknown origin, whereas splenectomy in connection with operations for malignant neoplasms predominates.

The surgical indications (Table IV) are dominating, as already stated, by the traumatic ruptures. These are analysed in Table V. They differ from the splenectomy material in general by the fact that they occur mainly in children and young

Table V. *Traumatic rupture of the spleen*

	Men	Women	Total
Traffic accidents	17	8	25
Fall	8	3	11
Stab wounds	2	—	2
Iatrogenic splenoporto-graphy	2	—	2
Unknown origin	2	—	2
Infectious mononucleosis	1	—	1
	32	11	43

23/43 (53 %) of the patients were below 20 years of age.

Table VI. *Postoperative complications*

	No. of pats.
Prolonged fever periods	5
Bronchopneumonia	4
Bilateral pleural effusions	1
Extensive wound infection	1
Intraabdominal bleeding – relaparotomy	1
Suture insufficiency – relaparotomy	1
	13 (7%)

No postoperative deaths attributable solely to splenectomy

people—53 % are below 20 years of age. Furthermore, ruptures are much more common in males (74 %) than in females (26 %). Traffic accidents, most of them bicycle or motor-cycle accidents, predominate as the cause of the rupture, but it ould be noted that there are a number of in which children have been hurt by climb- and falling. The high frequency of splenic rupture in children and young people can probably not be due solely to the fact that they expose themselves to more risks than do adults, but might also be due to the spleen being softer and/or the elasticity of the thoracic cage greater among children than among adults, so causing the spleen to be compressed and ruptured.

Other surgical indications for splenectomy are almost exclusively connected with surgical interventions for other reasons. The spleen is removed in radical operations for malignant tumours in adjacent organs (25 %) or because it has been unintentionally damaged during an operation (18 %).

The increasing interest in splenectomy as a therapeutic measure, therefore, concerns mainly the medical indications.

Haemolytic anaemia

The most well established indication, hereditary spherocytosis, has occurred in 10 patients. Other forms of haemolytic anaemia constitute a group in which it is often difficult to decide whether to perform splenectomy or not. Two patients may illustrate this.

Case 1

A 54-year-old man, hospitalized because of fatigue, jaundice and fever of few days duration. He was found to be severely anaemic and jaundiced with Hb 4.5 g/100 ml and bilirubin 8.8 mg/100 ml. An intense erythropoietic activity compatible with a haemolytic condition, was seen in the bone marrow. He was treated with prednisolone and ACTH without effect. A splenectomy was performed on almost vital indications with an immediate improvement, his Hb rose to 12.8 g/100 ml within 4 days. His prednisolone dose was gradually tapered off, which resulted in new haemolytic attack, starting some 5 weeks after the splenectomy. This time he was given prednisolone and azathioprine with good result. The Hb values rose to 14 g/100 ml with a normal number of reticulocytes (0.1–0.2 %). He was taken off steroids 6 months later and off azathioprine after 12

Table VII. *Follow-up study of patients with thrombocytopenia and haemolytic anaemia*

	Patients (total)	(traced n)	Healthy	Dead	Continued haemolysis
Thrombocytopenia	17	13 (77 %)	13[a]		
Haemolytic anaemia					
Hereditary spherocytosis	10	8	8		
Other forms	14	9	5	2[b]	2
Total	24	27 (71 %)			

One man, aged 29 had an acute relapse 2 years after splenectomy. Rapid effect of short-term cortisone treatment.
One woman, aged 21 3 years after splenectomy gave birth to a child with congenital thrombocytopenia, which spontaneously disappeared after 3 weeks.
One man, aged 50, dead from aplastic anaemia 4 years after splenectomy.
One man, aged 66, dead from prostatic carcinoma with metastases 1 year after splenectomy.

months, and has remained in an excellent condition with normal haematological values for 6 months without any medication.

Case 2

A 69-year-old man with previous history of various allergic manifestations, admitted because of the findings of dark urine and high ESR. He was found to have haemolytic condition with positive direct Coomb's test and an enlarged spleen, Hb 9 0 g/100 ml, anhapto-globinaemia, and reticulocytosis of 10%. Splenectomy was performed with good immediate result. Three weeks after the operation new attack of hyperhaemolysis occurred, more severe than before, with Hb 3–4 g/100 ml, and hyperbilirubinaemia (4–5 mg/100 ml). He was treated with prednisolone and azathioprine and after three weeks his Hb had risen to 11 12 g/100 ml and the reticulocyte values were normal. He was kept on the medication for three months, whereafter an attempt was made to discontinue steroids. His Hb values fell, and prednisolone was again given. One year after the splenectomy all medication has been stopped and he remains in excellent condition with normal blood values.

These two patients show interesting similarities. In both, splenectomy has been performed for acute haemolytic anaemia of unknown origin, the first patient being operated on for a vital indication. In both, a rapid improvement followed, accompanied by a recurrence of severely increased haemolysis 3–5 weeks later successfully treated by immunosuppression. It seems as if the splenectomy had triggered an immunological reaction, which worsened the already existing haemolysis. In this context it is of interest to note that syndromes resembling infectious mononucleosis have been reported in the postsplenectomy period and that these conditions have been thought to be due to damage to the immunological competence of the patient (19)

The experience with immunosuppressive treatment as a primary measure in haemolytic anaemia is restricted and it is impossible to tell whether in these patients one could have managed without splenectomy There are results in other diseases indicating that it is easier to handle immunological problems *after* splenectomy—e.g. it is possible to give compatible platelet transfusions with much better results (11)

Studies with radioactive isotopes to analyse the site of maximal erythrocyte breakdown seem to be a valuable preoperative procedure (9)—a high isotope uptake over the spleen speaks in favour of splenectomy

Thrombocytopenia

Seventeen patients have been splenectomized because of essential thrombocytopenia. A recent study (2) has demonstrated that a comparatively small proportion of all patients with severe thrombocytopenia have been splenectomized. Steroids had been the most common form of treatment, given to 43% of the patients, and only 19% had undergone splenectomy in many cases after unsuccessful steroid treatment. Steroids seem to be the method of choice if a definite response is not obtained within a few months, or the doses have to be kept so high as to produce a Cushing state, splenectomy should be performed.

As in haemolytic anaemia, isotope studies ha e been useful also in thrombocytopenic purpura to determine the probable benefit from splenectomy A good correlation was found between high splenic sequestration of platelets and the short and long-term results of splenectomy while with a high hepatic sequestration splenectomy was followed by a complete failure in 70% (16)

Malignant lymphoma

Malignant lymphoma is one of the fields where the interest in splenectomy is especially great. In early cases of e.g., lymphatic leukaemia such a large number of the pathological cells may be found in the spleen that splenectomy brings a definitely diminished load on the body often leading to prolonged remissions, as in the following patient

Case 3

A 57 year-old woman complained of an abdominal tumour and was found to have an enlarged spleen. Fine-needle biopsy and bone marrow examination demonstrated that she suffered from lymphatic leukaemia rih moderately high degree of cellular differentiation The peripheral blood picture, including differential WBC, was normal. Therapy was started with irradiation of the spleen, resulting in severe temperature reaction hh considerable deterioration of the general condition I was decided not to continue irradiation but to perform splenectomy The operation was performed in March 1969 A histological study of the spleen confirmed the diagnosis of chronic lymphatic leukaemia. The patient is well 2 / years after the operation and has had—or has—no signs whatsoever of her malignant lymphoma.

In more advanced stages of lymphatic leukaemia the postoperative complications are especially numerous (5). Splenectomy is often performed to alleviate an existing anaemia, especially of the

haemolytic type. It is difficult, however, to know in advance whether the "hypersplenic" inhibition of cell production is limited only to one cell line or affects all to the same degree. The following case may illustrate this.

Case 4

A 42-year-old man, who at a regular check-up was found to have an elevated ESR, was further examined and found to have splenomegaly and peripheral WBC of 65 000 with 99% lymphocytes. No anaemia, no thrombocytopenia. He was treated with splenic irradiation with good result and remained well for 15 months, after which time his blood values rapidly deteriorated with anaemia (Hb 7.5 g/100 ml) and thrombocytopenia (50 000). He sustained a splenic infarction and splenectomy was performed. His WBC, which before the operation was 22 000, rose to a level of 100 000–150 000 and has remained there since. His Hb rose somewhat but has remained low; his platelet values rose after the operation and have remained at 100 000.

The discussion of splenectomy in Hodgkin's disease has been especially lively during the last few years. It has been stated that only after laparotomy and splenectomy is it possible correctly to establish in what stage of the disease the patient is and thus to make estimates of prognosis and choice of therapy (8). Further, it has been emphasized that the susceptibility to cytostatic drugs would increase after splenectomy (14).

[Apl]astic anaemia

[Apl]astic anaemia is another interesting indication.

Case 5

A 25-year-old female psychologist, admitted with aplastic anaemia (Hb 6.1 g/100 ml, WBC 2 300, platelets 12 000). She had previously been healthy. A few months before she fell ill she had taken a few tablets of an amphetamine preparation, otherwise no cause for her anaemia could be found. She was given prednisolone and testosterone without effect and later a bone marrow transfusion from her brother was tried. Nothing influenced her aplastic anaemia and she had to be given regular blood transfusions, in all 74 units during 10 months. The course was complicated by intraocular bleedings, that made her almost completely blind. When nothing else seemed to help, splenectomy was performed, with an immediate effect on the platelets, which rose from a low of 3 000 to a level of approximately 100 000–200 000. The bleeding symptoms ceased promptly. Only a few units of blood were given after the splenectomy to correct the postoperative anaemia. In retrospect, it is probable that the eyesight of the patient could have been saved, had the splenectomy been performed earlier.

Japanese experiences speak in favour of splenectomy in patients with aplastic anaemia (12). It seems to be of interest to try the method more systematically in patients with otherwise therapy-resistant aplastic anaemia (21).

In the present material complications have been few. Seven patients have succumbed to their primary malignant disease within weeks or a few months after the operation, and 2 patients have died postoperatively after extensive gastric operations including splenectomy. Thus splenectomy was in itself performed without postoperative mortality. Postoperative complications were registered in 13 patients (7%) (Table V). Two of the infectious complications, one pneumonia and one wound infection, occurred in patients with lymphatic leukaemia.

Follow-up

A limited follow-up has been performed for patients splenectomized for thrombocytopenia and haemolytic anaemia. Due to the fact that many of the older medical records are stored in central archives and cannot be reached without great difficulty, it has not been possible to get information complete enough to trace all patients.

Thrombocytopenia. Thirteen patients out of 17 (77%) have been reached. All were healthy—2 to 21 years postoperatively—with normal platelet values. One man, aged 31, reported that he had had an acute attack of thrombocytopenia 2 years after his operation. No cause was found and the platelet values returned to normal after a short treatment with prednisolone. One woman, aged 27, three years after her splenectomy when her own platelet values were normal, gave birth to a child which was found to be severely thrombocytopenic. The platelet values, however, spontaneously returned to normal within a few weeks.

Haemolytic anaemia. Seventeen patients have been traced. All the patients with hereditary spherocytosis were well. Among 9 patients with haemolytic anaemia of unknown aetiology 5 (56%) reported that they were well with normal blood values, 2 still had haemolysis and were treated with cortisone and 2 had died in diseases probably connected with their initial haemolysis. Thus, approximately half of the patients with haemolytic anaemia of unknown origin benefited from the splenectomy.

Unfortunately it is impossible from this study

to form any definite policy regarding splenectomy The operative risk is small, especially if splenectomy is regarded as one of several therapeutic measures already in the primary evaluation of the patient and not saved as a last resort. Continued clinical studies of the course in splenectomized patients are necessary and a prospective splenectomy register as has been created in Gothenburg, is of special interest.

It should be emphasized that with transfusions of fresh whole blood and/or platelet-enriched plasma the bleeding during operation can always be kept under control.

The decision to perform splenectomy must be made by physicians and surgeons who have personal experience of the situation, and should be based on clinical evaluations as well as on laboratory examinations, among which isotope studies to analyse the site and the degree of cellular sequestration (erythrocytes and platelets) seem to be of special value.

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Table I. *Oxygen consumption (VO), cardiac index (CI), stroke index (SI) and heart rate (HR) before (I) and after (II) therapy (n = 13)*

	Rest				Work					
	Supine		Sitting		300 kpm/min		600 kpm/min		900 kpm/min	
	I	II	I	II	I	II	I	II	I	II
VO (ml/min/m²)										
Mean			148.8	139.8	512.4	482.8	783.9	755.2	1 149.8	1 039.7
S.D.			20.4	19.8	37.0	53.4	88.4	58.5	166.3	85.7
CI (l/min/m²)										
Mean	3.72	3.33	2.95	2.52	6.06	5.36	7.61	6.96	9.09	8.80
S.D.	0.62	0.44	0.53	0.29	0.78	0.70	0.86	0.89	1.28	1.31
SI (ml/stroke/m²)										
Mean	50.7	49.9	39.4	36.5	58.0	54.5	60.4	57.1	59.3	58.7
S.D.	6.9	6.6	7.6	4.3	6.6	6.5	7.5	7.1	7.7	8.2
HR (beats/min)										
Mean	73.9	67.1	76.0	69.5	105.2	98.3	126.7	122.5	153.8	150.2
S.D.	12.2	6.5	12.4	8.3	13.3	9.8	14.2	13.7	16.8	16.1

11.30 a.m. The blood was centrifuged and stored in a deep frozen state. At the end of the study all samples were sent deep frozen to the Merck, Sharp & Dohme, Research Laboratory USA, without information about the patients' doses. The analyses were performed by J. E. Baer. The method consisted basically of protein precipitation with perchloric acid, alumina absorption and determination of the fluorescence of the iodine oxidation product from the acid eluate. The mean values of the two observations are used in the calculation.

RESULTS

Casual blood pressure

The casual BP dropped in all subjects during treatment, the mean values from 176/115 mmHg before start to 146/100 mmHg at the last routine control.

The hemodynamic data are shown in Tables I and II and Fig. 1

Table II. *Systolic (SAP), diastolic (DAP) and mean arterial pressures (MAP) and total peripheral resistance index (TPRI) before (I) and after (II) therapy (n = 13)*

	Rest				Work					
	Supine		Sitting		300 kpm/min		600 kpm/min		900 kpm/min	
	I	II	I	II	I	II	I	II	I	II
SAP (mmHg)										
Mean	157.8	138.8	165.7	150.2	189.8	169.0	196.5	176.8	214.2	197.8
S.D.	16.0	16.9	15.1	14.9	17.3	20.9	21.4	21.1	24.6	20.0
DAP (mmHg)										
Mean	95.8	84.1	102.9	93.1	106.9	93.6	105.4	95.0	113.9	103.5
S.D.	8.8	12.1	7.3	9.4	9.6	11.8	12.7	12.4	14.7	13.6
MAP (mmHg)										
Mean	119.3	105.5	126.0	114.3	139.7	125.6	143.4	127.9	152.5	141.1
S.D.	11.7	14.4	8.8	10.5	12.2	15.5	16.9	17.2	20.0	16.5
TPRI (dyn sec cm⁻⁵ m²)										
Mean	2 613	2 547	3 517	3 655	1 864	1 888	1 519	1 482	1 359	1 318
S.D.	357	301	607	443	277	198	200	224	209	249

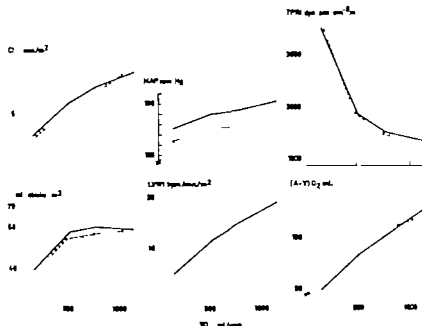


Fig 1 Hemodynamic changes at rest and during exercise before (---) and after ( ) treatment with α-methyldopa. Mean values. *LVWI* = left ventricular work index (calculated), (A V) O_2 = arteriovenous oxygen difference (calculated). Other abbreviations as in Tables I and II.

Oxygen consumption

The oxygen consumption (VO_2) at *rest* (sitting) was lower in 10 subjects at the second examination. The mean drop was 6% The difference between the mean values before and after therapy was, however not statistically significant. During *exercise* the VO_2 showed the same tendency as during rest. At the 900 kpm/min work level the mean reduction was 10% or 110 ml/min/m² (almost significant).

Cardiac index

At *rest* the cardiac index (CI) dropped in all subjects in the supine position and in 12 of 13 in the sitting position. The mean drop in the supine position was 0.39 l/min/m² or 10% (highly significant) in the sitting position 0.43 l/min/m² or 14% (highly significant) In the sitting position 9 subjects had a reduction in CI greater than 10% and the mean values dropped from 2.95 to 2.52 l/min/m² The difference between these mean values is almost significant. During *muscular exercise* the mean CI was lower after therapy than before, particularly at the two lowest work levels, the reductions being 12 and 9% and the mean changes significant. At the highest work level, however the reduction was insignificant, only 3%

Heart rate and stroke index

At *rest* the *heart rate* (HR) was reduced after therapy in 10 subjects in both positions, the mean reduction being 9% (significant) The difference between the mean values did not, however reach statistical significance in the supine nor in the sitting position. During *muscular exercise* the HR also tended to be lower after therapy but at the highest work level the reduction was only 2%

The changes in the *stroke index* (SI) were small, being at *rest* practically unchanged in the supine position, and in the sitting position reduced about 7% (insignificant) During *muscular exercise* the changes in the SI were insignificant. At the highest work levels the mean values were almost identical

Arterial pressure

The systolic (SAP), diastolic (DAP) and mean arterial pressures (MAP) were reduced after therapy at rest and during work, the reductions being significant. The changes in each of these three parameters were rather similar

At *rest* MAP was reduced 12% in the supine and 9% in the sitting position. In the sitting position MAP was reduced 10% or more in 6 subjects. Also the differences between the mean values of MAP at rest were significant both in the supine and in the sitting position.

Plasma concentration
µg/ml
30
20
10
500 1000 1500 mg
Daily dose

Fig Relationship between daily dose of α-methyldopa and plasma concentration.

During *muscular exercise* MAP was reduced in all subjects after therapy The reduction was most pronounced at the two lowest work levels, at which the mean reduction was 10 and 11% At the highest work level the mean reduction was 7%

ΔC
%
30
20
10
10 20 30 µg/ml
Serum concentration

Fig. 3. Relationship between serum concentration of α-methyldopa and changes in MAP sitting position at rest.

ΔMAP
%
30
20
10
10 20 30 µg/ml
Serum concentration

Fig 4 Relationship between serum concentration of α-methyldopa and changes in CI, sitting position at rest.

Total peripheral resistance index

Before therapy all subjects had an elevated total peripheral resistance index (TPRI) (at rest sitting > 2 700 dyn sec cm^{-5} m^{2}) The effect of therapy on the TPRI was, however disappointing. *At rest* the findings were inconsistent and the differences not significant in any body position. At rest, sitting, the TPRI *rose* in 10 subjects, and the mean change was a rise of 4%

During *muscular exercise* the effect upon the TPRI was also inconsistent. The mean changes were small, an increase of 1% at the lowest and a decrease of 2 and 3% at the highest work levels A decrease in the total peripheral resistance of at least 10% at rest sitting and during two work loads was not obtained in any patient.

Serum concentration of α-methyldopa

One subject had forgotten to take his morning dose on the day of the second study His serum concentrations were 0.01 and 0.00 µg/ml in the two tests. He is excluded from the calculations of the mean dose and mean concentration value. In the other 12 subjects the mean serum concentration was 1.04 ± 0.71 µg/ml. The dose/plasma concentration relationship is shown in Fig. 2. There is a clear tendency for the plasma concentration to increase with increasing dosages.

Fig. 3 shows the relationship between the plasma concentration and the changes in the MAP (in the sitting position). In spite of great individual

variations there is a positive correlation. The relationship between plasma concentration and the changes in CI (in the sitting position) is seen in Fig. 4. There is a positive correlation.

DISCUSSION

Most short-term studies on the effect of orally given α-methyldopa have shown a reduction in BP due to a reduction in total peripheral resistance, with no or only small changes in the cardiac output (CO) (1, 10, 11, 13). In some acute studies clear reductions in CO have been found (8, 10, 12, 14) but the drop in MAP has sometimes been relatively greater and, consequently, there has been a fall in the total peripheral resistance (8, 10).

In the light of these acute and short-term studies the results in the present study are surprising and disappointing. Both at rest and during exercise the drop in BP was associated with a drop in the CO and practically no change in the total peripheral resistance. The drop in the CO was mostly due to reduced HR.

The present study demonstrated a small drop in VO_2 at rest. Similar results were found by Sannerstedt et al. (11). The finding could be explained by the central nervous sedating effect of α-methyldopa (2, 9). During muscular exercise the VO_2 after treatment was also lower than before, particularly at the highest work level.

The study demonstrated a tendency to lower HR after therapy. Similar findings were made by Chamberlain et al. (1) and by Sannerstedt et al. (11). This finding could also be explained by the sedating effect of α-methyldopa (2, 9).

The drop in BP obtained with α-methyldopa in the dosages used was in general less than the drop obtained with 100 mg hydrochlorothiazide daily in a similar group of patients in a previous study in this laboratory (4). The mean casual BP dropped from 176/115 to 146/100 mmHg in the α-methyldopa group and from 180/113 to 140/95 in the hydrochlorothiazide group. The mean value for the MAP at rest sitting fell from 126 to 114 mmHg in the α-methyldopa group versus 131 to 107 in the hydrochlorothiazide group. Similar moderate BP reductions during rest were also observed by Sannerstedt et al. (11) (a mean drop in MAP of 16 mmHg). During severe muscular exercise the relative drop in MAP was small, in contrast to the results of Sannerstedt et al.

A striking feature in this study was that during submaximal exercise the effect of α-methyldopa was clearly smaller than at rest. The MAP was reduced only 11 mmHg and the HR, SI and CI were nearly unchanged. A possible explanation could be that the major hypotensive effect of α-methyldopa is centrally mediated as suggested in more recent studies (2, 9). During severe muscular exercise this effect could be drowned in the peripheral control of the cardiovascular system in this situation.

Most of the subjects in this study complained of tiredness, even after weeks on α-methyldopa, and it was a striking contrast between these frequent complaints and the total absence of complaints of side-effects in the similar group of patients treated with hydrochlorothiazide. Nor did the present study demonstrate a general reduction in total peripheral resistance with α-methyldopa. It should be remembered, however, that the present study did not include any observations on the regional blood flow. Earlier acute studies have reported a reduction in the resistance in the cerebral and renal vessels (6, 7). Long-term studies are, however, lacking.

The main conclusion from this study is that, judged from the effect on the central hemodynamics, α-methyldopa does seem to reduce the BP mainly by a reduction in CO and with inconsistent or no effect on the total peripheral resistance. A more physiological pressure reduction is generally obtained by the use of hydrochlorothiazide in such patients. The undesired hypokalemia, which is the most common side effect with a thiazide preparation, can easily be controlled by a small dose of spironolactone (5).

ACKNOWLEDGEMENT

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QUANTITATION OF RED CELL CARBONIC ANHYDRASES B AND C AND HEMOGLOBIN F IN THYROID DISORDERS

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Abstract. Carbonic anhydrase (CA) B and C and hemoglobin F (Hb F) have been studied by immunoelectrophoresis in normal subjects, in patients with thyroid disorders and in women during pregnancy and treatment with oral contraceptives, altogether 93 individuals. A close negative correlation was found between the concentration of the erythrocyte CA B and the serum thyroxin concentration. In pregnancy and during contraceptive therapy however the CA B concentration was normal. CA C concentration was normal in all groups except in pregnant women, in whom a slight, but significant elevation of the mean concentration was observed. In thyroid dysfunction there was no significant alteration in the erythrocyte content of Hb F. The results—especially the value of CA B determination as a diagnostic tool—are discussed.

It has been shown by several authors that the concentration of human red cell carbonic anhydrase (CA) B is decreased in hyperthyroidism and increased in hypothyroidism (4–6, 7, 8, 9, 15, 17, 18). The concentration of CA C seems to be normal (4–6, 9). In some patients with hyperthyroidism the hemoglobin F (Hb F) concentration *in the red cells has been found to be in*creased (7). The purpose of the present investigation was to examine, by new immunochemical methods for determination of CA B and C and Hb F, the diagnostic value of these quantities. Because of the simplicity of the methods employed the analyses can easily be employed as routine laboratory analyses.

MATERIAL AND METHODS

Blood samples from 93 adults were obtained by venepuncture and collected in heparin for determination of CA B, CA C and Hb F. The material included: 1) 31 normal individuals matched according to age and sex to those with thyroid disease, 2) 20 patients with hyperthyroidism, 3) 11 patients with hypothyroidism, 4) 23 pregnant women and 5) 8 women taking oral contraceptives.

The diagnosis of thyroid disease was established clinically and with the aid of laboratory procedures. The latter included determination of plasma thyroxin (T_4) by a modification of the method of Murphy (11). Normal range was 40–140 nmol/l, analytical coefficient of variation 6.4%. The antigen standard, the antibodies and the immunoelectrophoretical determination of CA B and C were the same as described in a previous study (13). The normal ranges for mean concentration of erythrocyte CA B and C were 3.4–6.4 g/l and 0.46–0.74 g/l red cells, respectively. The analytical coefficients of variation determined as the day-to-day reproducibility were 2.8 and 5.5% for CA B and C, respectively. Separate determination of CA B could be carried out without the carbamoylation procedure (12).

The procedure was then as follows. The diluted samples and CA B standards were used without carbamoylation and immunoelectrophoresis was carried out in agar gel 1 mm thick, pH 8.6 at 10°C with 8 V/cm for 18 hours. The antibody concentration in the 1% agarose gel was 0.5% v/v (Anti Human Carbonanhydrase B Behringwerke, Marburg/Lahn). The packed red cells of the samples and a pool from 100 blood donors were diluted 240 times. The amount of trapped plasma was estimated to 6%. The pure CA B solution containing 1 g CA B/l (13) was diluted 1:40, 1:80, 1:160 and 1:320. The height of the rockets was measured after staining the precipitates with coomassie brilliant blue. If pure standard solutions of CA B and C are not available, a pool of packed red cells from normals may be used. The concentration in such a pool has been found to be 4.5 g/l B and 0.60 g/l C. Aliquots of such a pool may be kept at −20°C for at least one year without any appreciable decrease of the concentration of the enzymes.

The Hb F concentration was determined by a sensitive immunoelectrophoretical technique. Specific antihemoglobin F antisera were obtained by immunization of rabbits with a partly purified concentrated Hb F solution. The antisera were absorbed with an adult hemolysate with a very low Hb F concentration. Quantitative immunoelectrophoresis was carried out in a 1 mm thick, 1% agarose gel (antibody concentration 1% v/v) at 10°C

Table I. *Erythrocyte CA B and C and Hb F concentration in various states*

Mean = arithmetic mean. Range = extreme range

		Erythrocyte CA (g/l)					
		CA B		CA C		Hb F (%)	
	n	Mean	Range	Mean	Range	Mean	Range
Hyperthyroidism	20	2.19	1.20–3.27	0.53	0.37–0.79	0.42	0.01–1.10
Hypothyroidism	11	6.07	5.01–8.30	0.57	0.43–0.77	0.07	0.01–0.26
Pregnancy	23	4.45	3.10–5.10	0.73	0.60–0.83		
Oral contraceptives (oestrogen + progesterone)	8	4.50	4.00–5.80	0.56	0.46–0.78		
Normals	31	4.45	3.30–5.50	0.58	0.42–0.82	0.13	0.01–0.41

with 5 V/cm for 18 hours. A standard solution of Hb F prepared from cord blood containing 1 g/l of Hb F was used in dilutions as follows: 1:20, 1:40, 1:80 and 1:160. The packed red cells from adults were diluted in demineralized water 1:3 and 1:30. The Hb concentration in these dilutions was determined and the fraction of Hb F could then be calculated. The Hb F concentration in the cord blood was determined as alkali-resistant Hb by the method of Betke et al. (3). Both in the CA B and Hb F quantitation exactly 2 μl of the dilutions of samples and standards are filled in to the holes before electrophoresis.

RESULTS

The concentrations of CA B and C and Hb F in the different groups are shown in Table I. Se thyroxin (T_4) was measured in 20 patients with hyperthyroidism, in 11 patients with hypothyroidism and in 6 normal euthyroid individuals. The corresponding values for CA B and T_4 are shown in Fig. 1.

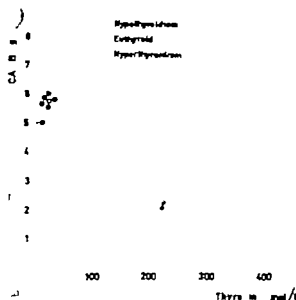


Fig. 1. Correlation between erythrocyte CA B and T_4 ($r = 0.90$; $p < 0.01$).

As the results shown in Table I may not be normally distributed, the non-parametric method of Mann-Whitney was used for significance testing.

When matched normal individuals are compared to patients with thyroid disorders, the CA B concentration of patients with hyper- and hypothyroidism is found to be significantly lower and higher respectively ($p < 0.01$). This was not the case for the CA C concentration. During administration of oral contraceptives and in pregnancy the concentration of the CA B enzyme remains within the normal range, whereas during pregnancy the CA C is slightly but significantly increased ($p < 0.05$).

When comparing the Hb F concentration of the 31 normals with the Hb F values of the 31 patients with thyroid disorder a slight but significant difference was found ($p < 0.05$). The average Hb F concentration was higher in thyrotoxicosis and lower in hypothyroidism, although several patients in both groups had a normal Hb F concentration.

During treatment of the thyroid disease the concentration of both CA B and Hb F changes to normal values. Fig. 2 shows two patients with hyperthyroidism and one with hypothyroidism before, during and after treatment.

DISCUSSION

The logical way of estimating the activity of the thyroid gland is to determine the concentration

of biologically active (i.e. non-protein-bound) thyroid hormones (triiodothyronine and thyroxin) to which tissues are exposed. This is not possible. Therefore other parameters are studied, such as the concentration of total thyroid hormone in plasma and the thyroid uptake of radioiodine, or even quite indirect indices, such as the basal metabolic rate. However the results of all these tests may be modified by a variety of non-thyroid conditions. Most important is that the estimation of T and protein-bound iodine (PBI) may be misleading in pregnancy and in women taking hormonal contraceptives. Thus in many cases, and above all when investigating pregnant women or women on oral contraceptives, a reliable parameter of thyroid function is needed. Therefore the concentration of erythrocyte CA B and C has been studied not only in thyroid disorders but in pregnancy and during administration of oral contraceptives as well.

Weatherall and McIntyre (17) found a decreased concentration of CA B in hyperthyroidism and an increased concentration in hypothyroidism. This has been confirmed by several others (4 6, 7 8 9 18) Funakoshi and Deutsch (4) using a quantitative immunodiffusion method, could not verify increased values for CA B in hypothyroid patients. In the present study we have found a significantly lowered CA B concentration in all patients with hyperthyroidism, all patients being below the 95% normal range. In the 11 patients with hypothyroidism the values for CA B were slightly but significantly higher than in the 11 matched controls. However all the values of the hypothyroid patients except two were within the 95% normal range determined in a previous study (13), which comprised apparently healthy blood donors.

In megaloblastemic anemia high concentrations of CA B have been described (15) CA B determination is thus of limited value as a parameter in hypothyroidism. Normal CA B values were found in pregnant patients and in women during oral contraceptive therapy Probably the increased values of CA B in pregnant patients obtained by Funakoshi and Deutsch (4) are due to the slight hypochromic anemia in these patients, because the values were expressed as the concentration of CA B in mg/g Hb. They also found a slight increase in the concentration of CA C in pregnant patients.

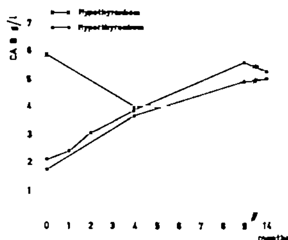


Fig. 2 Erythrocyte CA B before, during and after treatment in two patients with hyperthyroidism and in one patient with hypothyroidism.

The results of this study confirm that the concentration of CA C is normal in patients with thyroid disease. The cause of increased values in pregnant patients needs further investigation.

The Hb F concentration has been found slightly increased in some patients with hyperthyroidism and lower than normal in some patients with hypothyroidism. During treatment of the thyroid disease the abnormal Hb F values changed to normal values. The highest Hb F concentration observed (1 10%) in one of our patients with hyperthyroidism is considerably lower than the highest concentration (up to 20%) reported by others (7)

In thyroid disease both the bone marrow and the peripheral blood undergo changes (1 2, 5 16). In hyperthyroidism the bone marrow is hypercellular (1). In the peripheral blood an increased osmotic fragility (2, 15) and a shortened erythrocyte survival time (10) ha e been observed. The high concentration of glucose-6-phosphate dehydrogenase (G-6-PD) is well established (16) Furthermore the red cell sodium is increased (5). The opposite changes, although less marked, are observed in hypothyroidism (16, 17)

Erythrocytes from patients with hyperthyroidism have several features in common with fetal erythrocytes in cord blood. The fetal erythrocyte has low CA B content (< 1 0 g/l) increased G-6-PD and high Hb F concentration.

The biological role of CA B is still unknown.

The above mentioned inverse relationship between CA B and G-6-PD raises the question whether some interaction takes place between these two enzymes.

The commonly employed tests in evaluating the activity of the thyroid gland may occasionally be difficult to interpret. Therefore CA B determination as a simple laboratory analysis may be a helpful diagnostic tool, enabling with a high degree of accuracy the distinction of hyperthyroid from normal subjects. Its value, however, is less in the distinction between hypothyroid and euthyroid subjects, but it must be realized that this parameter is totally empirical and indirect and in this respect less satisfying.

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THE PRIMARY CAUSE OF RHEUMATOID ARTHRITIS IS AN INFECTION —THE INFECTIOUS AGENT EXISTS IN MILK

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Abstract. The theory of an infection as the primary cause of rheumatoid arthritis (RA) has been discussed periodically since more than four decades. The present author has made investigations on this problem over period of many years. Several clinical symptoms, particularly as regards the findings in the joint fluid and in the granulation tissue, speak in favour of this opinion. Experimental research on animals with the aim of provoking an arthritis similar to RA has lent considerable support to the theory of an infection as the basis for RA. In my opinion the arthritis provoked by type of Diplostreptococcus agalactiae belonging to the streptococci group B constitutes the condition of experimental arthritis most similar to human RA. In this respect it is important to consider that the experimental arthritis provoked by agalactiae is combined with the production of hemagglutinating macroglobulin which, with our present methods, cannot be differentiated from the human rheumatoid factor (RF) W have long been engaged at our Institute in research into the source of infection. In our opinion it is question of milk infection and, as most milk consumed is pasteurized, the infection for the most part derives from pasteurized milk. I this connection it is important to emphasize that agalactiae cocci are resistant to the temperature used for pasteurizing milk. Furthermore, agalactiae can be cultivated from the nasopharynx in about 75–80% of RA cases. All RA patients investigated showed antibodies to agalactiae cultivated from the nasopharynx and from milk as well. Experimental arthritis and rheumatoid-factor-like macroglobulin can be produced by the agalactiae cocci cultivated from pasteurized milk.

My coworkers and I have for more than three decades focused our interest on investigations of the etiology and pathogenesis of rheumatoid arthritis (RA). The experimental work has mainly followed three lines:

1. Studies of experimental arthritis (20, 21 23 27).

2. Bacteriological and immunological studies in patients suffering from RA and in experimentally produced arthritis (23 27).

3. Studies of the rheumatoid factor (RF), its nature occurrence experimental production and cleavage products (24 25 26, 28)

In this paper a new concept will be treated, i.e. bacteria in milk as cause of rheumatoid arthritis.

MATERIAL AND METHODS

For production of experimental arthritis several methods have been tried in our laboratory but the principal one has been injections of Diplostreptococcus agalactiae (DSA), cultivated from the nasopharynx or in some cases from human intestinal content. Mucus taken with platinum loop from the nasopharynx is inoculated into medium of 1% galactose broth and simultaneously inoculated directly on 1% galactose agar with 5–8 horse blood. The inoculated media are left at 34°C for about 24 h, after which recultivation is done on galactose blood plates. The cultures can be kept alive for years in tubes with 1% galactose agar to which 5% horse serum is added. After addition of serum these tubes are heated to 90°C and thereafter allowed to congeal in oblique position. Cultures from feces were made by the same method.

For animal experiments 1–2 platinum loops of living bacteria from 4–48 h blood or serum agar are emulsified in 2 ml 0.9% NaCl and injected into white rats (Sprague-Dawley) intraperitoneally intravenously or intracutaneously or in the epiphyseal plate and its neighbourhood. In our experiments the intraperitoneal route was mostly used, and sometimes, in addition, injections were made in the epiphyseal plate of knee. (This kind of injection was tried since we had observed in autoradiographic experiments that the RF had strong affinity for the epiphyseal plate.)

Injections are given on 5 consecutive days and one injection 3–4 weeks later If the results as regards formation of RF are found to be poor injections are given every 3rd–4th week until reaction is obtained.

Blood for the hemagglutination test (Waaler-Rose) is taken from the experimental animals about 4 weeks after the first injection and 1 2 months later etc.

Biopsies are made for studies of the joint capsule joint fluid.

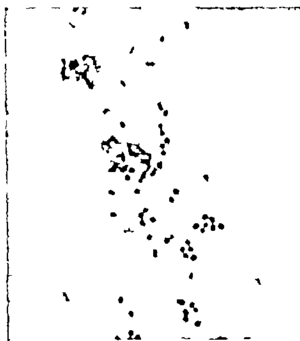


Fig 1 DSA in direct smear from human nasopharynx.

As regards the animal experiments, the best results have been obtained when using white rats. Pigs also respond well to injections of DSA, but of course rats are much easier to handle. Guinea pigs should not be used, since untreated animals of this species often show a positive sheep cell test. Rabbits are excellent for production of arthritis but, peculiarly enough, they do not give rise to haemagglutinating macroglobulin, or only to very weak one. Sheep and dogs do not give satisfactory results.

Typing of DSA with antisera has been done, using the ...fied Lancefield method. Different types of antisera been used, but mostly antisera group B from ...Werke and from Difco, Detroit. Control tests to be made with antisera groups A and D (enterococci). Precipitation has never been obtained with antisera group A, but cross-reactions often occur with antisera group D

It should be added that DSA usually does not form chains, or only short ones, even in liquid medium. Therefore the name Diplostreptococcus is justified (Fig. 1).

A most important test is the determination of the resistance of the agalactiae cocci to heat. The resistance to heat has been tested using graduated, electrically heated water baths. The influence on the agalactiae has been routinely tested at 70, 80 or 90°C during 10 min (+1 min equilibration).

The sensitivity to different pH values has been measured using 1% galactose broth acidified to pH 1.5 2.0, 2.5 3.0 and 4.0 with HCl. The pH meter used was E396 Metrohm. The tubes have been kept at 34°C during 18–24 h.

Special bacterial properties of less importance for the problems discussed in this paper are not described here, since this was done in earlier reports (23). This applies, e.g. to the facultative anaerobic growth of agalactiae and the resistance to bile (30–40%) and the tendency for pleomorphism.

A series of experiments has been concerned with cultivation of DSA from milk, particularly pasteurized milk, (heated to 72°C, 20–30 sec) i.e. the kind of milk which is mostly used at present. Some cultivations have also been made from cream containing 12% or 40% fat, heated to 80–85°C, 20–30 sec. Two platinum loops and, to gain further experience, 0.1–0.2 ml of the material have been inoculated into tubes containing 1% galactose agar with horse serum, or sometimes in Todd broth or on the type of blood agar plates described above. The milk material is taken under sterile conditions directly from tetrapak containers. If growth of different kinds of bacteria is obtained, new inoculation is made with heating of the inoculated tubes to 75°C for 10 min, thus taking advantage of the high degree of thermoresistance of DSA, which seems to be almost unique for vegetative forms of bacteria.

DSA isolated from milk often has kind of diffuse capsule. This property diminishes slowly after a number of recultivations. The DSA from milk has given unusually high titres on agglutination particularly in the first period after isolation. W have formed the opinion that this strong agglutination with milk bacteria is per haps due mainly to the above mentioned forming of capsules.

Intracutaneous tests on RA patients have been made with vaccine of agalactiae by the commonly used methods.

Comprehensive trials have been made to reveal the presence f agglutinins against agalactiae in human sera. Both living and killed cocci were used. Four different strains of agalactiae were employed. The human sera were diluted with 0.9% NaCl. The final dilutions after addition of bacteria were 1 16, 1 32, 1 64 etc. The tubes were left for 2 hours at 37°C and overnight at about 20°C

RESULTS

Diplostreptococcus agalactiae, belonging to group B streptococci, has been obtained in pure culture from the human nasopharynx and from feces. From the nasopharynx it could be isolated in 75–80% of cases of rheumatoid arthritis and in 20% of persons with other diseases and healthy individuals. Sometimes these cocci have been obtained directly in pure culture from the nasopharynx in RA. They have always been pleomorphic and in diploform. The growth of DSA is facultative anaerobic and is often considerable in 40% bile.

DSA has also been isolated from pasteurized milk. The reason why these bacteria remain in milk in spite of pasteurization is their high resistance to heat. They always resist 70 C for 10

Table I. *Heat resistance of DSA (10 min) in broth*

+ = growth, 0 = no growth

	70°C	80°C	90°C
Isolated from human beings			
E_7 (RA)[a]	+	+	0
KJ (RA)	+	+	0
GE (colitis)	+	+	+
Isolated from milk			
MJ	+	+	+
W	+	+	0
Bo	+	+	0
Lo	+	+	0
P	+	+	+
F	+	+	0
D	+	+	0

The 7-sign means the 7th isolation of agalactiae from one and the same patient.

Table III. *Cultivation of agalactiae from milk*

Sweden	+
Denmark	+
Norway	+
France	+ (slight growth)
Germany	+
England	+
Canada	+
USA	+
Sweden, cream, 40%	0

min and often 80 C or even more (Table I) Some strains are able to sustain 85–90 C, and it happens that isolated bacteria even sustain a short stay at 100°C. They are except enterococci to my knowledge the only vegetative bacterial forms that have about the same thermoresistance as spores. This circumstance, of course highly facilitates the isolation of DSA.

In contrast to its heat resistance DSA is very sensitive to acids. Table II shows two examples of this. E_7 is a strain from the nasopharynx of a patient suffering from RA, and GE a strain from feces. The E_7 cocci do not even sustain glucose broth with pH 4 while the other strain grows at this acidity The acidity was produced with HCl as reported under Methods.

The normal value of HCl in the gastric juice is pH 1–1.5 This acidity probably inhibits the development of DSA. The bacteria survive better in hypochlorhydria, a condition common in RA. However it is not quite clear when hypochlorhydria starts in RA, whether it exists before the appearance of the disease or not

Table II. *Resistance of agalactiae to acidity*

	pH					
	1.5	2	2.5	3	3.5	4
E_7	0	0	0	0	0	0
GE	0	0	0	0	0	+

The cocci in question give rise to hemolysis on blood agar plates with 1% galactose. The degree of hemolysis varies greatly The hemolysis assumes a brownish colour (methemoglobin) In the drinking of milk a great number of hemolytic DSA enter the gastrointestinal tract. When mixed into food many probably survive the gastric acidity

Returning to the presence of DSA in milk, this coccus has hitherto been found in all specimens of pasteurized milk from Sweden (some 20 specimens) and in specimens from Denmark Norway France Germany England, Canada and USA (Table III) In France and Sweden the coccus was found in pure culture from pasteurized milk. One or two platinum loops of milk gave usually rise to 200–800 colonies, i.e. millions of bacteria. The milk bacteria are hemolytic, but often somewhat less so than from human beings. The colonies are initially thicker and denser than those obtained from the nasopharynx. The DSA from milk is also thermoresistant. Specimens from other countries, except France and Sweden, ha e shown 1–2 other contaminants, but this condition requires study of a still larger number of specimens. Only from one country were there spore-bearing rods in the milk. Clostridia were not found. It is remarkable that the French pasteurized milk ga e rise only to one colony of DSA on cultivation from one platinum loop This is the same finding as for 40% Swedish cream which is long-time pasteurized and practically sterile, while 12% cream shows the same bacteria as pasteurized milk.

The typing of the milk cocci showed that they belong to agalactiae. Table IV shows the grouping of DSA in milk as well as of DSA from human beings. This Table has a particular interest since it shows that three of the strains isolated from

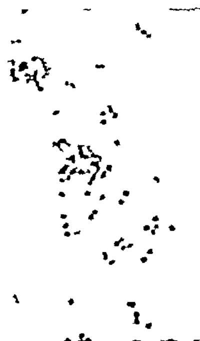


Fig. 1. DSA in direct smear from human nasopharynx.

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RESULTS

Diplostreptococcus agalactiae, belonging to group B streptococci, has been obtained in pure culture from the human nasopharynx and from faeces. From the nasopharynx it could be isolated in 75–80% of cases of rheumatoid arthritis and in 20% of persons with other diseases and healthy individuals. Sometimes these cocci have been obtained directly in pure culture from the nasopharynx in RA. They have always been pleomorphic and in diploform. The growth of DSA is facultative anaerobic and is often considerable in 40% bile.

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Table VII. *Agglutination of 80 human sera with strains obtained from human nasopharynx* (H) *or from pasteurized milk* (M)

The different tests are divided into groups according to the degree of agglutination of the H bacteria. Thus the agglutination with milk bacteria and the sheep cell test are made on the same group of sera as those indicated with H

RA			Other collagen diseases			Non-collagen diseases			Healthy		
H	M	Sheep cell	H	M	Sheep cell	H	M	Sheep cell	H	M	Sheep cell
0–1:128	0–1:2 048	0–1:4 096	0–1:128	0–1:4 096	0–1:4 096	1:32–1:128	0–1:8 192	0–1:64	1:8–1:128	1:32 1:4 096	0–1:32
1:256–1:1 024	1:1 024–1:4 096	1:8 192–1:16 384	1:512–1:1 024	1:256–1:16 384							
1:2 048–1:8 096	1:8 096										

of cocci. Thus, even strains not resistant to heat have been called Streptococcus fecalis.

The DSA from milk provokes in rats an RF like macroglobulin and slight arthritis, as was found on many occasions with agalactiae from RA. Table V shows an earlier demonstrated experiment for provoking RF-like macroglobulins with agalactiae isolated from an RA patient. Males reacted less than females. Table VI shows production of hemagglutinating macroglobulin with DSA. Further results will be published later.

I now pass over to series 2, bacterioimmunological studies, mentioned in the introduction.

Intracutaneous injections of killed DSA were performed several years ago. The reactions showed considerable variations. Sometimes a strong delayed reaction was observed with erythema 2–3 cm in diameter and a papula with edema and infiltration 1.5–2 cm in diameter; sometimes there was only a slight erythema. The reaction remained for 1–2 days. There was no definite parallelism between the seriousness of the RA and the degree of intracutaneous reaction. A positive reaction occurred in about 55% of the patients, while in healthy individuals the test was mostly negative or weak but sometimes also definitely positive. The test was made with DSA isolated from human nasopharynx. However, the test did not give much valuable information.

Several series of agglutination tests between different strains of agalactiae and human sera have been made. The last series (Table VII), containing 80 different sera, was performed during the autumn of 1971 and spring of 1972. The test has been made with at least two different strains and often with four strains of DSA, two from RA patients (E_T and RA), one (GE) from a patient suffering from colitis, and one (MJ) isolated from Swedish pasteurized milk. Living DSA has been used, but as regards the GE strain parallel tests have sometimes been made with living and with killed DSA as well. The agglutination tests with killed DSA have usually shown the same results as those with living bacteria.

It was obvious that different DSA strains gave rise to a dissimilar degree of agglutination at different occasions, as is often the case with other species of bacteria as well when, e.g. the recultivations had been irregular.

As may be seen from Table VII, the strain isolated from milk often gave rise to higher agglutination titres than DSA from nasopharynx. This might possibly be due to the circumstance that the milk bacterium had a kind of capsule. There was no parallelism between the bacterial agglutination and the sheep cell test. The strains were not agglutinated by isolated RF.

The variability of DSA is rather pronounced. Thus the degree of hemolysis is more pronounced in some strains than in others, but the hemolysis also varies in one and the same strain. This happens when it has not often been re-inoculated. There are also variations of the heat resistance. Particularly important is that some strains are agglutinated both by anti-B and anti-D serum. I am aware of the fact, that different types of DSA may be involved. On the other hand one and the same strain may show variability in this respect, too. I am not prepared to give a definite answer on this question. However, all of the strains are more or less capable of giving rise to RF and arthritis.

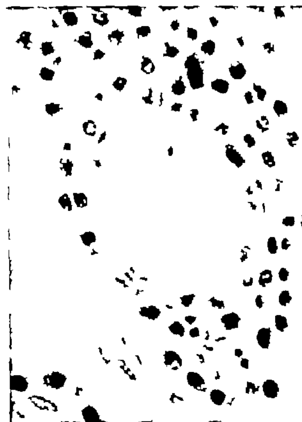


Fig 2 Experimental arthritis provoked by DSA. Perivascular granulation tissue.

As regards the third type of research touched upon at the beginning of this paper it has concerned experimental investigations of the RF The method for isolation of RF and the character this hemagglutinating macroglobulin have been (22, 23 27) Only one point should be ...ed here. The experimentally provoked like macroglobulin contains a hemagglutinating subgroup belonging to the 7S globulins, similar to that of the RF macroglobulin in human beings (28) The same is the case with human hemagglutinating macroglobulins in other diseases than RA while non-hemagglutinating macroglobulins, e.g. the macroglobulins in healthy individuals, never contain such a hemagglutinating subgroup. This group can be completely separated from common 7S.

CONCLUSIONS AND SUMMARY

As pointed out before, rather many authors are nowadays of the opinion that the basic cause of RA is an infection In an early time the common streptococci A were discussed as etiologic agents (3 10, 14 16). In the last decade interesting research has been done on mycoplasma and some other micro-organisms (8 13)

For many years I have made studies with DSA (20). However I have hesitated to claim that DSA is the primary cause of RA. The reason why this idea is now presented is that numerous important facts have appeared during the last years.

First some notes about the question whether DSA is able to provoke disease. This bacterium was usually considered to be harmless to human beings, but it was known to give rise to mastitis in cattle. For a long time we have discussed the presence of DSA in the human body In the last six years also several other authors have, however expressed some doubt about the opinion that DSA would not give rise to human disease. This has not been discussed in connection with RA by other authors, but DSA has been found, for example in the vagina as a cause of vaginitis, from which may also derive infection of newborn infants (1 11). As regards the presence of agalactiae in the pharynx they were earlier probably mixed up with cocci belonging to group A or D since DSA has not been described However on cultivation from the nasopharynx DSA seldom gives rise to chains, or only to short ones even in liquid medium which already in the beginning of my studies brought me to doubt that it was the question of streptococci belonging to group A.

Concerning the significance of DSA for RA, I would like to lay stress upon the following circumstances.

The cocci in question are found in the nasopharynx about four times as often in RA as in healthy persons. DSA is not seldom found in pure culture or with only a few other bacteria from the nasopharynx in RA, while this does not seem to occur in healthy individuals.

There is often a high titre of agglutinins against DSA from human nasopharynx in the serum from RA patients. The same is the case with DSA from milk If the agglutination test (agalactiae + human serum) is performed with DSA isolated from milk, positive tests with high titre rather often occur also in other diseases than RA and in healthy persons (as shown in Table VII). The significance of this has to be investigated on a still larger material.

A strong cutaneous reaction against killed DSA is rather common in RA and under all circumstances much more common than in healthy individuals.

As regards the animal experiments, it has been cogently established that both an RF-like macroglobulin and arthritis can be provoked by injections of DSA in animals (e.g. white rats) either through intraperitoneal, intravenous, intracutaneous or other types of injections. The RF-like macroglobulin in animals cannot, with our present methods, be distinguished from the human RF and it has the same hemagglutinating subgroup as human RF. The two RF macroglobulins have the same sedimentation constant, but their molecular structures are not known. Of course, there could be some difference in the sequence of amino acids, but small dissimilarities are in this case of no real importance considering that the main properties are the same and that differences in the amino acid sequence often occur between, e.g., the light chains in human beings suffering from one and the same disease (15).

The granulation tissue in RA patients and in experimental arthritis is of the same type: thickening of the walls of the vessels with proliferation of the fibroblasts, a great number of lymphocytes and plasma cells, but no polynuclear leucocytes and no eosinophils (Fig. 2).



Fig. 4. Experimental arthritis in rat provoked by injections of DSA.



Fig. 3. Joint fluid two days after injection of DSA in rabbit. Diplococci are seen, as well as polynuclear leucocytes. The cocci are sometimes accumulating on the surface of the cells (in the middle).

As in human beings the joint fluid, in contrast to the granulation tissue, contains mainly polynuclear leucocytes (Fig. 3). The presence of DSA in the joint fluid during the first days after injection is surely caused by the sudden massive infection in connection with the injection. I should mention that a large number of mononuclear cells in the fluid after 3–4 weeks is more common in animals than in human beings. After some weeks, scarification of the granulation tissue begins, with appearance of abundant fibroblasts (23).

The arthritis in animals, as in humans, is slowly progressive and the changes are often symmetrical. X-ray examinations reveal decalcification and erosions, narrowing of the joint space and formation of osteophytes (Fig. 4).

As is well known, an RF-like macroglobulin may be provoked by other bacteria than agalactiae, e.g. typhoid bacilli, but here arthritis is lacking. On the contrary other micro-organisms, e.g. mycoplasma, are able to provoke arthritis but do not give rise to RF.

I would like to stress once again that DSA

gives rise both to definite arthritis and to RF experimentally

As regards the part of the body constituting the entrance for the infection, there are several possibilities. Most often probably there is a proliferation of bacteria in the nasopharynx but it might also be in the intestine. It has not been possible to follow in detail the route of infection in the body of human beings, but in this respect it is interesting to study the development in experimental arthritis. If e.g. DSA is injected intraperitoneally or intravenously into white rats, there is a short period of what might be called sepsis. Thus, for 1 or 2 days bacteria are found in the joint fluid, as well as abundant polynuclear leucocytes (Fig. 3) A few cocci-like inclusions are seen in the leucocytes and besides, on the surfaces of cells as well as extracellular. The cocci disappear after 2–3 days.

After about 3–4 weeks a granulation tissue appears in or in the neighbourhood of the joint capsule. It consists of perivascular proliferation of the fibroblasts. Around the vessels there appear a large number of lymphocytes and plasma cells but no polynuclear leucocytes (23 27). After some months a scarification of the nodules usually takes place.

At about the same time as the granulation tissue is produced a hemagglutinating macroglobulin of type RF is formed, i.e. 3–4 weeks after the first bacterial injection.

It is not quite clear how the RF-like macroglobulin is formed. There are several possibilities. The infectious agent and its metabolic products might stimulate a genetically preexistent globulin in a lymphocyte and bring it to increased production. Or the infectious agent stimulates the formation and liberation of enzymes, e.g. in the lysosomes of macrophages and gives rise to metabolic products stimulating the production of macroglobulins, either influencing genetically preexistent macroglobulins of RF nature or provoking changes of the normally occurring macroglobulins. The RF-like macroglobulin exists both in the granular tissue and joint fluid and in the cells of this fluid, plasma cells, but also lymphocytes and polynuclear cells (4 9 13 22)

It is known that in connection with these processes immune complexes are formed in the cells of the joint exudate and in the granulation tissue (e.g. Hollander et al. (9)). Immunological processes are certainly underlying some of the clinical findings in RA.

Some authors have emphasized that, if an environmental factor is the primary cause of RA this factor must exist all over the world, since RA has an ubiquitous spread. As regards the investigations reported here, the agalactiae cocci seem to fulfil these demands. We have cultivated agalactiae from pasteurized milk from several European countries and from the USA and Canada (Table III). The reason why we have made investigations on pasteurized milk, is, that this is the kind of milk that is commonly used at present and, besides, it is much easier to isolate agalactiae from pasteurized than from non-pasteurized milk, which contains a large number of other bacteria.

As a matter of fact almost all human beings consume milk, but why do not all of them get RA? In this connection we have the same reason to ask why does not everybody in groups eating the same food infected with typhoid or dysentery bacilli, fall ill in these diseases? I want to draw attention to a few reasons why everybody does not contract infections which are very wide spread. Many have a "natural" resistance to infections, probably due mainly to a good immunological defence. Another reason for dissimilarities is the fight between different kinds of bacteria. Thus, new micro-organisms often do not be able to proliferate in a part of the body where already vigorous bacteria exist

As far as the stomach is concerned, the degree of acidity is important. Bacteria sensitive to acidity like agalactiae are often repressed by normal acidity or hypersecretion of the stomach. Also other conditions may as is known play a role, but the above remarks will perhaps suffice in this connection since similar questions have been discussed many times also with reference to other diseases.

It is a well known fact that other bacteria than those mentioned may cause human arthritis. Postinfectious arthritis is, e.g. an accepted term. But also in RA different bacteria may play a role. Everybody knows that RA may take a more serious course after for instance, a secondary angina, pneumonia etc.

Cecil (3) pointed out many years ago that it took 30 years before it was commonly accepted that rheumatic fever was caused by streptococci

belonging to group A. It is now more than 30 years since the infectious theory of RA began to receive recognition. Many circumstances seem to favour the opinion that Diplostreptococcus agalactiae plays a decisive role in the pathogenesis of RA.

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Congress Announcements

The Fourth International Congress on Lymphology will be held in Tucson Arizona, USA March 6–10 1973.

Information. Dr Ch. Witte, Dept. of Surgery University of Arizona College of Medicine, Tucson Ariz. USA.

The Second International Symposium on Cancer Detection and Prevention will be held in Bologna, April 1–1., 1973 under the auspices of the International Union against Cancer (UICC) and the International Agency for Research on Cancer (IARC) of the WHO

The Symposium is planned to discuss and evaluate the latest results in the field of cancer detection and prevention. Immediately following the Symposium, two days will be devoted to workshops on methods of prevention detection and diagnosis of cancer Scientific exhibits.

Secretary general. C. Maltoni, Belgium.

Secretariat: Istituto di Oncologio F Addarii" Viale Ercolani 4/2, 40138 Bologna, Italia.

The Second European Conference on Internal Medicine will be held in Bonn-Godesberg, West Germany May 3–5 1973

Organizer: The European Association of Internal Medicine (AEMIE).

Subjects of discussion. 1) Intensive care and emergencies in internal medicine inside and outside the hospital. 2) Immunology and internal medicine. 3) How should postgraduate internal medicine be taught?

Information. Dr J G L. Dagnelie, Secretary Association Européenne de Médecine Interne d'Ensemble, rue des Eburons 75 B-1040 Bruxelles, Belgique

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HARALD ASTRUP SALVESEN
IN MEMORIAM



Professor Harald Astrup Salvesen, M.D. Oslo Norway died on January 22, 1972, 83 years old.

Following training as an intern at the University Hospital, Rikshospitalet, in Oslo he studied physiology at the Institute of Physiology in Oslo in 1917. In 1918–19 and 1922–24 he worked in the USA mainly at the Rockefeller Institute in cooperation with Van Slyke. On his return from the USA he was resident at Medical Department B Rikshospitalet in 1924–28, then as chief of the Medical Outpatient Clinic at the hospital until he was appointed Professor of Medicine at Medical Department B in 1932. He worked here for 27 years.

In all these years he fulfilled the duties which rest on a clinical professor of medicine: research, teaching and patient treatment.

His research extends over a wide field of clinical and experimental medicine. His doctoral thesis from 1923 "Studies on the physiology of the parathyroids" was an experimental work. He maintained for the rest of his life an interest in the function of the parathyroids and calcium metabolism in, among other diseases, renal rachitis and sprue, a disease in respect of which he made contributions to its pathophysiology and clinical picture. His research was founded on his biochemical training and on an open clinical mind. He was able to see new manifestations of diseases and to make new correlations of symptoms and signs. In Boeck's sarcoid he described four manifestations which had not been observed earlier: hyperglobulinemia, opticus atrophy, myocardial disease with atrioventricular block, and kidney manifestation of that disease.

He was interested in kidney diseases and especially in renal acidosis. He clarified acid base disturbances in renal insufficiency. He also introduced alkaline treatment in this disease.

In liver diseases he observed disturbances in serum proteins with reduction of the albumin/globulin quotient, an observation which was verified during the hepatitis epidemic during the second world war. He was also interested in heart diseases. Thus he observed functional valvular insufficiency in uremia, a phenomenon still concerning us today and for which there is no satisfactory explanation.

As a teacher Harald Astrup Salvesen was loved and admired by a whole generation of Norwegian physicians. In his teaching he mainly stressed the demonstration of clinical manifestations of the disease. He discussed diagnosis and treatment on the basis of clinical findings. He was a gifted teacher who could arouse an interest for clinical medicine in his students. He always drew upon medical history and tried to compare new observations and new methods of treatment with those of earlier generations, but he also had an eye to the future. He looked forward to a more effective treatment of diseases. Already in 1932 he expressed this view: "The therapeutics have not followed up in the development of medicine today. Here one may in the future expect the

progresses to be made A prediction which has been fulfilled to a remarkable extent.

Personally Harald Astrup Salvesen was a lovely man. He tried always to see the good and the best in his fellow beings. Sometimes he was disappointed He then consoled himself with Kipling's "If"

If you can keep your head when all about you
Are losing theirs and blaming it on you,
If you can trust yourself when all men doubt you,
But make allowance for their doubting too,
If you can wait and not be tired by waiting,
Or being lied about, don't deal in lies,
Or being hated don't give way to hating,
And yet don't look too good, nor talk too wise.

Ole Storstein

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48-HOUR PRESERVATION OF PIG KIDNEYS USING CONTINUOUS HYPOTHERMIC PLASMA PERFUSION

Effect and Complications

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Abstract. Preservation of pig kidneys for 48 hours has been studied using continuous hypothermic plasma perfusion as preservation method. Twenty-six experiments were performed and the material was divided into two groups. In group 1 (10 experiments) the kidneys were perfused with a constant non-pulsatile flow (0.2 ml/g/min), which gave a perfusion pressure from 10 to 30 mmHg during most of the perfusion. In group 2 (16 experiments) a pulsatile perfusion pressure of about 65/35 mmHg was used, which gave a flow rate of 1.2 ml/g/min during most of the perfusion. One animal in group 1 survived an experimental period of 3 months and five died due to renal failure without signs of surgical complications. Three died with thrombosis of the renal artery and one due to stenosis of the ureter with ureteral leakage. Four animals survived in group 2, while nine died due to renal failure without signs of surgical complications. Three died with thrombosis of the renal artery. Changes in the preservation technique in group 2 (different pulse curves, addition of certain substances to the perfusate medium and pretreatment of the animals with mannitol) were tried without success. The pathogenesis of the many cases of renal failures is discussed, and in group 2, in which the development of widespread necrosis in the kidneys was the most frequent complication to the preservation, the phenomenon was investigated further. On the basis of histological examination of the kidneys, together with analyses for protein in the urine produced during the perfusion and in the first hour after transplantation, it is concluded that damage to the vascular system arises during the perfusion, and probably causes total or partial infarction of the kidney after recirculation.

In earlier papers we have described our experiences with 24-hour preservation of pig kidneys, using hypothermic continuous serum and plasma perfusion (13, 14). In accordance with other workers (1, 2, 4, 18) we have found consistently good results using a perfusion technique closely similar to that originally described by Belzer et al. (2).

Attempts to prolong the time of preservation for more than 24 hours have been described by different workers (2, 4, 10, 19). Some have obtained consistently good results up to 72 hours in experiments with dogs (2, 4), whereas others have found some cases of severe damage to the kidneys after a perfusion time of 48 and 72 hours (10, 19).

It is the purpose of the present study to demonstrate our results with 48-hour preservation of pig kidneys with continuous hypothermic plasma perfusion.

MATERIAL AND METHODS

Twenty-six female pigs of the Danish Landrace breed, 4 to 5 months of age, and weighing 48 to 72 kg at the time of surgery were used. During the experimental period the pigs were fed with a standard fodder mixture (8) and unlimited quantities of water were permitted. The animals were weighed once a week, and the daily administration of fodder was calculated on the basis of the b.wt. In the time from transplantation until abundant urine production was observed, water was restricted and the animals were kept on a protein-restricted diet, to which [illegible] g $NaHCO_3$ was added daily.

Preservation of the kidney

The material was divided into two main groups.

In *group 1*, comprising ten pigs, the kidneys were preserved by means of continuous non-pulsatile hy

Table I. *Variations in the experimental conditions in group 2*

Group 2	No. of pigs	Perfusate medium	Pretreatment	Pressure curve
I	7	ACD-plasma	Heparin 5 000 IU/10 kg	Pulsatile roller pump
II	3	ACD-plasma	Heparin 5 000 IU/10 kg, mannitol 10 500 ml	Pulsatile roller pump
III	3	ACD-plasma additives[a]	Heparin 5 000 IU/10 kg, mannitol 10 % 500 ml	Pulsatile roller pump
IV	3	ACD-plasma	Heparin 5 000 IU/10 kg, mannitol 10 500 ml	Pulsatile piston-type pump

Hydrocortisone 100 mg, magnesium sulphate 4 mEq, insulin 80 U and dextrose .30 mg/l plasma (Belzer et al. (7)).

(8°C) plasma perfusion using fixed flow rate of 0.. ml g/min. In seven cases the plasma as stabilized with heparin and in three with citrate. Details concerning the apparatus and the perfusion technique has been described previously (13).

In *group 2* comprising 16 pigs, the kidneys ere preserved by means of continuous pulsatile hypothermic (8°C) plasma perfusion, using perfusion pressure of about 65 35 mmHg, hich gave flow rate of 1 ml g

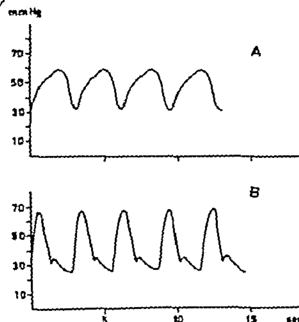


Fig. 1. The pressure in the renal artery during the perfusion using roller pump (*A*) and piston-type, positive-displacement pump (*B*).

min. In all cases ACD-plasma was used. Details concerning the apparatus and the perfusion technique are described earlier (14). The experimental conditions in group 2 varied ith respect to the pretreatment of the animals, the composition of the perfusate medium and the perfusion pressure curve as illustrated in Table I. The differences in the arterial pressure curve during the perfusion are shown in Fig. 1. In all cases samples of urine produced 1 2, 24 and 48 hours after the start of the perfusion ere examined quantitatively for protein.

Surgical technique

Renal autotransplantation with uretero-ureteral anastomosis was performed followed by simultaneous contralateral nephrectomy. Details of the surgical technique have been published previously (16).

Peroperative studies

Thirty minutes after recirculation, renal blood flow as measured by means of the Xenon-133 wash-out technique in eight cases in group 2. The technique has been described previously (12). One hour after recirculation biopsy was taken from the kidney in all cases. In 1 cases in group 2, J[125] albumin 20 mCi was given i.v. to the animals 10 min before recirculation of the transplanted kidney. The urine produced during the first 15 min after recirculation was collected. Blood was drawn from the pig during this period and 5 ml plasma together ith 5 ml urine as counted in an auto-gamma-spectrometer (Pacard). Urinary protein from the same period was determined quantitatively and examined by paper electrophoresis.

Postoperative studies

Blood analyses. During the first eek after surgery blood samples for creatinine determination were taken every other day and subsequently once ek for the following 3 months.

Kidney function. The clearances of inulin and para-aminohippuric acid (PAH) were determined on the 10 h, 31st and 94th days after transplantation in the surviving animals. In the last experiment the maximal tubular excretion (Tm) for PAH was determined. Three da) after the last clearance experiment the percentage ex traction of PAH was determined, after hich the animals were killed. Details of the doses of the test substances, the technique and the calculation methods ere as accordance to Gyrd-Hansen (8).

Analytical methods have been described previously (9).

Postmortem examinations. The animals which survived during the hole observation period ere killed and bled, and postmortem examination as performed. The animals hich died from complications ere examined as soon as possible after death.

Histological examination. At necropsy kidney tissue was removed and fixed in neutral buffered formalin. The biopsies, taken one hou after recirculation, were f xed in Zenker' fluid and in neutral buffered formalin. Paraffin wa sections re stained ith iron haematoxylin-van Gieson, and the periodic Schiff reaction as carried out according to McManus and Mowry (15). Fibrin stain according to Lendrum (17) as used in some cases



Fig. 2. Histological section of kidney biopsy from pig 102 (group I) 1 hour after recirculation. Proximal tubules with pycnotic nuclei (*a*) desquamation (*b*), granular PAS-positive material (*c*) and lack of brush border (←). Slight dilation of glomerular space. Zenker fluid. PAS. × 195



Fig. 3. Histological section of kidney biopsy from pig 144 (group 2 II) 1 hour after recirculation. Proximal tubules with intact brush border and protein (*a*) or PAS-positive material in the lumen (*b*). Zenker's fluid. PAS. × 195



Fig. 4. Histological section of the kidney from pig 142 (group 2 II) at autopsy 1 day after transplantation. Necrotic area of the cortex. Several small arteries with PAS-positive deposits in the lumen and in the wall. Neutral buffered formalin. PAS × 78

Table II. *Number of survivors and failures in groups 1 and 2*

Group	No. of pigs	No. of survivors	Thrombosis of renal artery	Necrosis of renal cortex	Death in uraemia with urine formation	Other complications
1	10	1	3	2	3	1 stenosis of ureter with ureteral leakage
2 I	7	3		2		
2 II	3			3		
2 III	3		1	2		
2 IV	3	1		2		

RESULTS

Group 1

The perfusion. Using a flow rate of 0.2 ml/g/min the arterial pressure was initially between 15 and 55 mmHg. During the first 3–8 hours of the perfusion the pressure decreased to values between 15 and 30 mmHg. It remained at this level for the rest of the experiment except in four cases, in which a moderate increase was noted after 35 to 40 hours. No urine production was observed during the perfusion.

Initial behaviour. Most of the kidneys turned pink after recirculation, but after 5–15 min all except one developed shifting blue areas on the surface and many became flaccid in consistency. Urine production was noted in all cases within min after recirculation. Histological examination 1 hour after recirculation revealed a picture as exemplified in Fig. 2. No pathological changes were found in the glomeruli. In the proximal tubules 70 to 75% of the nuclei of the cells were pycnotic, and moderate to abundant cellular desquamation was seen. Varying degrees of protein cylinders and PAS-positive granules in the tubules, interstitial oedema and focal leucocytosis were found.

Subsequent function. From Table II it can be seen that only one animal survived in group 1. In this case serum creatinine rose to 175 μg/ml and then fell below 20 μg/ml within 14 days after transplantation. Table III gives the values for the clearances of inulin and PAH and for the effective renal blood flow 10, 31 and 94 days after transplantation. Table IV shows the Tm of PAH on the 94th day after transplantation.

Postmortem examination. The kidney of the

Table III. *Average renal clearances 10, 31 and 94 days after transplantation*

For corresponding renal clearances in pigs with an autotransplanted not long-term preserved kidney see Gyrd-Hansen et al. (9)

Days after transplant.	Group	Pig. no.	B. wt. (kg)	Hct (%)	Diuresis (ml/min)	Inulin clearance (ml/min/10 kg b.wt.)	PAH clearance (ml/min/10 kg b.wt.)	Effective renal blood flow (ml/min/10 kg b.wt.)
10	1	98	52	32	1.1	8	35	56
	2 I	106	57	38	0.9	13	35	61
		107	60	30	3.1	8	41	64
		111	48	22	2.4	2	12	17
	2 IV	153	73	32	3.2	10	63	100
31	1	98	71	36	1.6	19	67	114
	2 I	106	83	44	2.4	24	63	122
		107	74	37	2.7	17	60	104
		111	61	32	1.7	9	32	51
	2 IV	153	88	32	1.5	19	81	128
94	1	98	118	40	3.1	19	58	105
	2 I	106	113	44	1.2	21	64	1 4
		107	110	43	2.9	21	48	92
		111	94	42	2.2	10	32	60
	2 IV	153	108	39	1.4	18	71	127

Table IV *Clearance and Tm of PAH 94 days after transplantation*

For corresponding values in pigs with an autotransplanted not long-term preserved kidney see Gyrd-Hansen et al. (9)

Group	Pig no.	B. wt. (kg)	Kidney wt. (g)	Relative kidney wt. (%)	Plasma PAH concentration (μg/ml)	PAH clearance (ml/min/10 kg b.wt.)	Inulin clearance (ml/min/10 kg b.wt.)	Tm (mg/min/10 kg b.wt.)	Tm (mg/min/100 g kidney)
1	98	118	345	0.29	1 520	26	15	17	58
2 I	106	113	470	0.42	1 050	36	19	18	42
	107	110	431	0.39	1 270	27	15	15	39
	111	94	337	0.36	1 600	16	8	12	34
2 IV	153	108	374	0.35	1 070	29	15	15	43

pig which survived during the entire experimental period revealed a normal macroscopical appearance. Microscopically a moderate interstitial fibrosis was found. Among the remaining nine animals three cases with complete arterial thrombosis were found. Two cases revealed a nearly total necrosis of the renal cortex without thrombosis in the renal artery. Four kidneys, which all produced urine before death, revealed the following changes. The macroscopical picture showed only moderate changes consisting of oedema and a variable number of yellow-white areas in the cortex. The microscopical picture showed necrosis of many tubules between the medullary rays, some of them with fragmented basophilic and PAS-positive cytoplasm. The remaining tubules showed vacuolated cytoplasm and PAS-positive cylinders. Interstitial fibrosis was present and in one case basophilic regenerating tubular epithelium.

Group 2

The perfusion. A constant pulsatile pressure of about 65/35 mmHg was aimed at in all cases and the flow rate was varied accordingly during the perfusion. Due to a decrease in the vascular resistance during the first 3–6 hours of the perfusion, the flow rate was gradually increased and was between 1–2 ml/g/min during most of the perfusion. However a slight increase in the vascular resistance during the last 5–10 hours was observed in ten cases. The concentration of protein in the urine during the perfusion is shown in Table V. It can be seen that a marked increase occurs in the period from 4 to 48 hours after the start of the perfusion.

Initial behaviour. Most of the kidneys turned pink after recirculation, but only one remained unchanged in colour during surgery while all others developed shifting blue areas on the surface and some became very cyanotic. Those with a good postoperative kidney function showed only minor changes in colour. The urine production started in all cases immediately after recirculation, and was often abundant in the first 10 to 15 min, but then decreased in varying degrees during the surgery. It was always possible to demonstrate coagulated protein in the urine collected during the first 30 min. In two cases a protein concentration of 37 and 38 g/l with a relative distribution of 51 and 57 % albumin and 49 and 43 % globulin, respectively was demonstrated. I. injected J[1] albumin in two cases showed a high concentration in the urine (56 513 and 64 4 1 cpm) as compared to plasma (84 531 and 97 837 cpm) *indicating that the protein in* the urine originated from glomerular filtration of plasma proteins in the transplanted animals. Renal blood flow 30 min after recirculation, which was measured in eight cases (group II, III,

Table V *Protein concentration (g/l) in urine produced during the perfusion (group 2)*

Pig no.	Group	Protein concentration At start	After 4 h	After 48 h
144	II	0.3	3.0	38.0
145		0.3	0.9	9.4
146	III	0.6	0.5	5.7
147		0.8	0.8	47.0
149		0.9	_4	8.5
151	IV	0.7	—	4.7

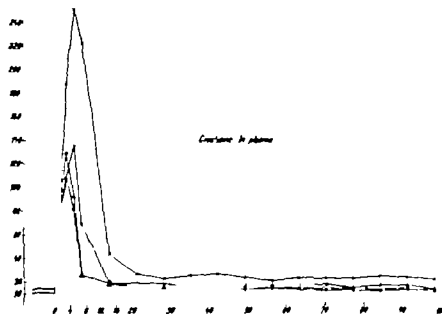


Fig 5 Concentration of creatinine in plasma of the four surviving animals in group 2. Ordinate: creatinine in plasma (μg/ml), abscissa: days after transplantation. ●—● = pig 106, O—O = pig 107 — — = pig 111 Δ—Δ = pig 153

IV) showed a mean value of 50 ml/100 g/min ± 15 (S.D.). Histological examination 1 hour after recirculation revealed a picture as exemplified in Fig. 3 Using iron haematoxylin–van Gieson and the periodic Schiff reaction no pathological changes were found in the glomeruli. In 12 cases no pathological changes in the cells of the proximal tubules were seen and in the remaining four it was only possible to demonstrate pycnosis of the nuclei in 2–10% of the cells. A dominating feature was a high incidence of tubules with PAS-positive material in the lumina. This was found in all cases and sometimes up to 50% of the tubules were filled with this material. Using Lendrum's fibrin stain all the cylinders stained as fibrin, and furthermore moderate deposits of fibrin were observed in the walls of the small arterioles, the capillaries of the glomeruli and in the cells of the proximal tubules.

Subsequent function From Table II it is seen that arterial thrombosis developed in three cases In the remaining 13 cases nine pigs died with nearly total necrosis of the renal cortex while only four pigs survived throughout the experimental period. Fig. 5 shows the concentration of creatinine in plasma (μg/ml) of the four surviving pigs, and Table III gives the values for clearances of inulin and PAH and for the effective renal blood flow 10 31 and 94 days after transplantation. Table IV shows the Tm of PAH on the 94th day after transplantation The extraction percentage for PAH was on an average 85 varying from 74 to 90.

Postmortem examination The kidneys from the four pigs which survived throughout the whole experimental period showed macroscopically varying degrees of fibrosis, especially in pig 111 which had 30–40% fibrotic tissue but otherwise the kidneys were normal. Microscopically the picture showed varying degrees of interstitial fibrosis but otherwise normal conditions. Thrombosis of the renal artery was seen in three cases In the remaining nine cases widespread necrosis of the renal cortex was seen without any signs of thrombosis in the main renal arteries (Fig. 7).



Fig 7 Macroscopical picture of the kidney from pig 144 3 days after transplantation, showing typical renal cortical necrosis as seen in nine cases in group 2.



Fig. 6. The same kidney as in Fig. 4. Lumen of an arteriole with leucocytes and erythrocytes (yellow). Fibrinpositive material both in the lumen and in the muscular layer between the muscle cells. Lamina is visualized as thin blue ring. Neutral buffered formation. Lendrum. 244.

Histological examination was carried out in seven of these cases and in five a microscopical picture as shown in Figs. 4 and 6 was seen. The most striking feature was seen in all the small arteries and arterioles in the cortex, where PAS-positi e homogenous material was deposited both on the intima and in the tunica media between the muscle cells. Often the lumina were totally oc cluded (Fig. 4). In many arteries the intima was absent. In two cases these vascular changes could not be demonstrated. A great number of PAS-positive cylinders were found in the collecting tubules in all cases. Using Lendrum s fibrin stain the PAS-positive material stained as fibrin (Fig. 6).

DISCUSSION

The results show that the preservation techniques in use could only in a few cases preserve pig kidneys for 48 hours without serious damage.

The perfusion technique used in group 1 with a low fixed flow rate (0.2 ml/g/min) and conse quently a low perfusion pressure was not suc cessful in 48-hour preservation Only one animal survived throughout the experimental period. Three pigs died uraemic, but with abundant urine formation, while two died without any urine production and with widespread necrosis of the renal cortex. Four animals died due to surgical complications (Table II) This is in accordance with our earlier experiences with this technique in 24-hour preservation experiments (13). A possible explanation could be an insufficient per fusion in parts of the kidneys, an assumption which is supported by the fact that the oxygen consumption in dog kidneys, perfused under hypothermic conditions with cell-free media, increases with increasing flow rate when this is below 1–1.5 ml/g/min (7) When compared to group 2 it was characteristic that the histological picture of the kidneys in group 1 1 hour after recirculation, showed a much higher degree of cellular damage in the tubules and that the histological picture at the time of necropsy showed no specific vascular lesions as found in group 2.

The perfusion technique used in group 2, with a pulsatile perfusion pressure of about 65/35 mmHg and a flow rate of 1–2 ml/g/min, has revealed consistently good results in experiments with 24-hour preservation of pig kidney (14). This is in accordance with the results of other workers, using dogs as experimental animals (1 2, 4 18). On this account the method has been used in the clinic, at the present time mostly with a time of perfusion less than 24 hours (3 5) The results seem somewhat varying, but the varying conditions of the kidneys at the start of the per fusion may have played an important role. Thus Belzer et al. (3) ha e shown that vascular spasms which develop in the agonal phase reduce the possibility of a successful result, even when the kidney has a short time of warm ischaemia and a short tim of perfusion If the period of pre servation does not exceed 24 hours, the con tinuous plasma perfusion must compete with the much simpler storage technique using hypother mic storage after a short initial cooling perfusion with a perfusate medium which mimics the intracellular ion composition, first described by Collins et al. (6) This technique has been shown to preserve dog as well as pig kidneys (6, 12) for 24 hours, and is successful in pigs even with preceding warm ischaemic period of 1 hou (11)

In group 2 the postoperative behaviour of the kidneys was characterized either by relatively quick return to almost normal function (Fig 5) or by such serious damage to the kidneys that the possibility of reversible function could be ex cluded (Fig. 7) Four animals survived and nine developed widespread necrosis in the renal cortex, without signs of thrombosis in the main renal arteries. The development of this serious complication was not correlated to a specially high increase in the vascular resistance during the perfusion. Changes in the pulse curve (Fig. 1), addition of hydrocortisone, magnesium, insulin and dextrose to the perfusate as recommended by Belzer et al. (2) and pretreatment of the animals with mannitol did not prevent the development of this complication.

Concerning the lesions found in group 2 the following facts ha been noticed. 1) Nearly no pathological changes were found either in the glomeruli or in the cells of the tubules in the biopsies taken 1 hour after recirculation using haematoxylin-eosine and PAS staining methods (Fig. 3). A high frequency of PAS-positive cylinders was found in the tubules and collecting ducts in all the biopsies. The use of a more specific fibrin staining method (Lendrum) indicated that these cylinders are fibrin, and further more revealed moderate fibrin deposits in the

small arterioles and glomerular capillaries.) At the histological postmortem examination of the kidneys which developed cortical necrosis after transplantation a high frequency of fibrin cylinders was still found in the tubules and the collecting ducts. In these kidneys, furthermore, nearly all the arterioles and small arteries had their walls and lumina filled with fibrin diminishing or occluding the lumina of these vessels (Fig. 6). 3) Measurements of renal blood flow 30 min after recirculation revealed a low flow (50 ml/100 g/min ± 15 S.D.) as compared with non-preserved kidneys (153 ml/100 g/min ± 81 S.D.) and 24-hour preserved kidneys (130 ml/100 g/min ± 45 S.D.) measured with the same technique (14). 4) The glomeruli showed increasing permeability for protein, especially at the end of the perfusion (Table V). This permeability was still found during the first period after recirculation when the plasma proteins from the recipient were found in high concentrations in the urine from the transplanted kidney.

On the basis of these findings it is concluded that the limiting factor for the preservation time in group 2 was damage to the vascular system during especially the last 24 hours of the perfusion. Due to increasing permeability of the of the arterioles and capillaries, the plasma invade the walls and pass through the capillaries. This may be fatal after recirculation when intramural and intraluminal coagulation occurs in the damaged vascular bed, leading to narrowing or total occlusion of the lumina of the renal arterioles.

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POTENTIAL SOURCES OF CADAVERIC KIDNEYS FOR TRANSPLANTATION IN A GENERAL HOSPITAL

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Abstract. An investigation, based on autopsy findings, has been made of the number of theoretically available cadaveric kidney donors among 1 400 patients dying in 1968 at our 900-bed hospital. Patients on whom no autopsy was performed ere excluded from the investigation Criteria for exclusion are: age above 70 years, cancer septicaemia or proven bacteriaemia, active tuberculosis, systemic disease, renal disease, hypertension, and terminal hypotension exceeding one hour Among 1 18. patients dying from natural causes 45 suitable donors (3 8%) ere found. Out of 56 patients dying from accidents, suicide and homicide ten (18%) would have been suitable kidney donors. Although most of the donors in the group dying from natural causes were older than 60 years, histological changes due to aging were only slight. Donors suffering from renal disease, cancer or active tuberculosis could be excluded with reasonable certainty by clinical evaluation and macroscopical examination of the kidneys. The investigation shows that, although the percentage of theoretically available kidney donors among patients dying from natural causes is only 3–4% the great number of patients in this group makes it an important, albeit hitherto underestimated, source of cadaveric kidneys.

With the present rate of progress in cadaveric kidney transplantation a shortage of available kidneys may well prove a limiting factor to the transplantation programs. In order to determine the number of theoretically available donors of cadaveric kidneys at the Municipal Hospital of Copenhagen, a retrospective survey of the 1 400 patients who died during 1968 has been carried out. Among the 1 241 patients on whom an autopsy was performed, a minimum of 55 were acceptable cadaveric kidney donors.

MATERIAL AND CRITERIA FOR EXCLUSION OF DONORS

The Municipal Hospital of Copenhagen is 884-bed hospital with about 20 000 admissions per year. It has no departments of neurosurgery thoracic surgery or paediatrics. During 1968 exactly 1 400 patients died in the hospital, of whom 1 185 (85%) died from natural causes and had an autopsy performed at the hospital. In these patients the kidneys were examined both macroscopically and by light microscopy The remaining 15 patients included all those who died as result of accidents, suicide and homicide (177 patients). Fifty-six of these patients had an autopsy performed at the Institute of Forensic Medicine. As, in these patients, no consistent laboratory investigation or microscopical examination were made of the kidneys, they are not included in the group of patients ho had an autopsy performed at the hospital, but constitute separate group. For various reasons no autopsy as performed on 159 patients.

Our criteria for exclusion of donors are nearly similar to those defined by Calne (3), although for practical reasons the age limit and the accepted period of terminal hypotension had to be defined precisely Patients older than 70 years were excluded. So was everybody suffering from cancer except those with primary intracranial tumours. Septicaemia or proven bacteriaemia, active tuberculosis, and systemic disease also caused exclusion. In order to avoid renal disease, we required that the kidneys should be of normal size and appearance, and that the serum creatinine concentration should be normal. A history of previous renal disease, proteinuria, abnormal urinary sediment, or hypertension, constituted grounds for rejection. For terminal hypotension, an arbitrary limit of *1 hour* was set.

AUTOPSIES AT THE MUNICIPAL HOSPITAL OF COPENHAGEN (GROUP I)

The primary evaluation of this group was based on the a topsy findings. In those not excluded by this screening procedure, a further and more thorough evaluation based on the case reports and the laboratory investigations was carried out. Because of the predominance of relatively old patients, special care was taken to determine whether changes in renal histology due to physio-

Table I. *Age and sex distribution of 45 potential cadaveric kidney donors (group I)*

	Age (y.)			
	41–50	51–60	61–70	Total
♂	1	6	19	26
♀	4	3	12	19
	5	9	31	45

logical aging were so severe as to exclude the donors selected by patho-anatomical and clinical criteria.

Changes due to physiological aging of the patients were defined as: a) atherosclerosis of small and medium-sized arteries, b) glomerular fibrosis and hyalinization, c) tubular atrophy, d) interstitial fibrosis with lymphocyte and plasma cell infiltration and e) medullary interstitial fibrosis (5). The degree of change was graded as absent, slight, moderate or severe. To ensure a uniform evaluation, the histological specimens from the kidneys of the potential donors were all examined by one senior pathologist (Dr P. Christoffersen).

The final outcome was 45 acceptable donors, presented by age and sex in Table I. Two thirds of the donors were in the 61–70 years age group, and only five were younger than 50 years. Twenty-three of the 45 donors died from cardio-pulmonary diseases, with myocardial infarction as the single disease responsible for the greatest number of deaths (11 patients). Seven patients died from cerebrovascular insults, five from primary intracranial tumours, six from hepatic insufficiency and four from other diseases. In accordance with these primary causes of death, 35 of the donors died in medical wards and six in the Department of Neurology, while only two came from surgical wards and two from other wards.

In order to estimate the risk of accepting

Table II. *Degree of physiological changes of the kidneys due to aging correlated to age of donors*

	Age (y.)			
Renal changes	41–50	51–60	61–70	Total
None	3	4	14	21
Slight	2	5	16	23
Moderate	0	0	1	1
	5	9	31	45

Table III. *Causes of death for 56 patients with autopsy performed at the Institute of Forensic Medicine (group II)*

Cause of death	No. of pats.	No. of suitable kidney donors
Natural death	20	2
Accident	30	8
Suicide	3	0
Homicide	1	0
Unknown	2	0
	56	10

donors with undiagnosed renal disease, cancer or active tuberculosis, we re-examined the case histories, laboratory and autopsy findings of the 480 patients younger than 71 years in whom these diseases had been found during life or at autopsy.

There were 64 cases of renal disease. Four of them were unsuspected at the time of death. However, three had macroscopical changes that would have been visible on removal of the kidneys. The fourth, a 52-year-old woman, developed an acute tubulointerstitial nephropathy during the last few hours before her death. Her kidneys were of normal size and appearance at autopsy, but microscopy revealed the tubulointerstitial lesions.

None of the 216 patients with cancer would have been acceptable. Only three patients neither had a definite diagnosis nor were suspected of suffering from cancer, and these were excluded for other reasons.

Only two of the thirteen patients with tuberculosis had signs of active tuberculosis. In both, the diagnosis had been made before the patients died.

With most of the donors in the 61–70 years age group there was a certain risk of encountering severe changes of the kidneys due to accelerated physiological aging. However, only one donor had moderate changes due to aging, while 23 had slight and 21 no visible changes (Table II).

AUTOPSIES AT THE INSTITUTE OF FORENSIC MEDICINE (GROUP II)

Forty-six of the 56 patients were excluded because of age, case history or macroscopical changes found at the autopsy according to criteria stated. The causes of death for the 56 patients appear from Table III. More than half of

the patients—among the ten potential cadaveric kidney donors eight—died from accidents (Table III) Eight of the ten potential donors were between 21 and 50 years old and only one was above 51 years.

DISCUSSION

Forty-five potential cadaveric kidney donors were selected according to our criteria from a total of 1 185 patients who died and had an autopsy performed at the hospital in 1968. From our experience with group I patients, all of whom were submitted to an examination of their kidneys both macroscopically and by light microscopy we must conclude that, if the kidneys are of normal size and appearance on macroscopical examination, renal disease is unlikely Thus another ten potential cadaveric kidney donors originating from group II may be added, giving a total of 55 kidney donors. This, however must be regarded as the minimum number of theoretically available donors, since our criteria have probably been too rigid. Successful transplantations of cadaveric kidneys from donors older than 70 years (4) and donors with terminal hypotension for up to 12 hours (1 2) have been reported. If we accept an extension of the terminal hypotension period to 10 hours while still adhering to the rest of our criteria, this would mean a further 11 patients qualifying as potential cadaveric kidney donors. On the other hand many ethical and practical problems will limit the number of available kidneys. These limiting factors include interpretation of death criteria, consent from next of kin, tissue typing service, and co-operation between different departments. Furthermore the occurrence of multiple renal arteries may cause technical problems. This vascular anomaly is found with a frequency of about 20% (7)

Among the suitable kidney donors we have included six patients dying from hepatic coma. None of them had been tested for Australia antigen, a procedure which probably should be included in the screening of potential kidney donors. In two of these patients a "hepatorenal syndrome" occurred as a terminal event. However at autopsy the kidneys were of normal size and appearance, including normal histology The suitability of such kidneys for transplantation has been demonstrated by Koppel et al. (6).

Although our retrospective study was founded on autopsy findings, the results could be correlated to the case histories, clinical and laboratory findings. Even if autopsy findings had not been available, and we had had to rely on what was known about the patients when they died, 99 7% of the patients who were found unsuitable would still have been excluded. In spite of the old age of the majority of the donors, changes of the kidneys due to aging were only slight. The combination of clinical evaluation of the patient and macroscopical examination of the kidneys seems to offer a reasonable protection against donors suffering from renal disease, cancer or active tuberculosis.

The present investigation has shown that even excluding patients dying from accidents, a hospital with about 1 400 deaths per year should be able to provide a considerable number of cadaveric kidneys for a transplantation unit While in the group of patients dying from accidents 10–20% may be acceptable donors of cadaveric kidneys, only 3–4% of patients dying from natural causes are acceptable. However this smaller percentage is balanced by the greater number of patients dying from natural causes.

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CLINICAL VALUE OF SERUM B_{1A} AND B_{1E}-GLOBULIN LEVELS IN ADULT PATIENTS WITH RENAL DISEASE

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Abstract. Serum levels of B_{1A}-globulin, a metabolic product of the third component of complement, have been investigated by a simple immunochemical method in a group of adult patients with renal disease. A renal biopsy was performed in 100 of 105 adult patients with primary renal disease and in 27 of 31 patients with secondary or other renal disease, enabling classification on histological grounds. Decreased B_{1A} levels were found in acute post-streptococcal glomerulonephritis (APGN) and in some patients with membrano-proliferative glomerulonephritis (MPGN) and glomerulonephritis associated with systemic lupus erythematosus (SLE). In these three categories of patients B_{1E}-globulin, the fourth component of complement, was also determined by the same simple immunochemical method. In APGN B_{1A}-globulin rises to normal levels in 1–4 months after the onset of the disease. The B_{1E}-globulin is occasionally decreased at a very early stage in APGN, and then returns rapidly to normal levels. In MPGN the B_{1A} level may be constantly low for many years. Some patients, however demonstrate a change in the level with time. The B_{1E} level is usually normal in this group. A number of patients with MPGN had, at least on a single determination, normal B_{1A} levels. In SLE glomerulonephritis decreased levels of both complement components are found, the B_{1E}-globulin usually being diminished to a greater extent than the B_{1A}-globulin. Occasionally only the B_{1E} level is low which could mean that the determination of B_{1E}-globulin is of greater significance for the diagnosis of SLE than the determination of B_{1A}-globulin. Two patients with SLE showed a parallel increase in both complement factors during long-term treatment with large doses of corticosteroids. SLE differs in this respect from APGN, and this suggests a different manner of complement activation. From this group of adult patients it may be concluded that the findings relating to serum B_{1A} levels in children with renal disease are also applicable to adults. In addition, the determination of B_{1A}- and B_{1E}-levels by a simple technique is of value in the differential diagnosis of patients with glomerulonephritis.

Serum complement levels may be decreased in acute post-streptococcal glomerulonephritis (APGN) (5, 6, 7, 11, 12, 15, 21) in membrano-proliferative glomerulonephritis (MPGN) (1, 5, 6, 8, 15, 18, 19, 20) and in glomerulonephritis associated with systemic lupus erythematosus (SLE) (2, 5, 7, 10, 12, 14). The determination of total haemolytic complement (C'_{H50}) is rather impracticable for clinical use. Therefore the level of B_{1A}-globulin—a reaction product of B_{1C}-globulin, C'_{3}—is usually measured. Klemperer et al. (9) have shown that a decrease in B_{1A}-globulin in general parallels the decrease in the C'_{H50} titre in patients with renal disease.

A decrease in serum B_{1A}-globulin may be associated with low levels of other early-reacting complement components (5, 10). Gewurz et al. (5) investigated the complement profile in renal disease and found diminished levels of C'_{1} and C'_{4}, especially in SLE and to a lesser extent in APGN and MPGN while C'_{2} was mainly decreased in SLE and APGN but not in MPGN. Herdman et al. (8) also have described cases of chronic nephritis in whom the decrease of C'_{H50} titre was caused by a diminution of complement factors other than B_{1C}-globulin. As a result of these findings it seems worthwhile to determine not only the total complement level or B_{1A}-globulin in renal disease but also where possible other complement factors.

A group of adult patients with renal disease was investigated. Renal biopsies were obtained from nearly all patients, enabling a histological subdivision to be made. When a low B_{1A}-globulin level was found B_{1E}-globulin C'_{4}, was determined in addition. Recently it has become possible to measure B_{1E}-globulin as a routine test, like B_{1A}-globulin by using commercially available antiserum plates.

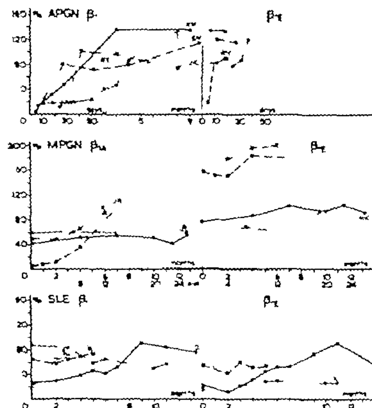


Fig. 2. Follow-up of B_{1A} and B_1 levels in patients with APGN, MPGN and SLE. Zero time in patients with APGN is the moment of first symptoms and signs (haematuria, oedema, hypertension).

In contrast to the course of APGN, the B_{1A} level remained low during a period of 2 years in two patients with MPGN; an increase was found in the two other patients (Fig. 2). There were no obvious histological differences between patients with a normal and those with a lowered B_{1A} level. Seven of the ten patients with a lowered B_{1A} level were young adults (aged 18–25); the remaining three were respectively 41, 47 and 65 years old. The average age of the patients with a normal B_{1A} level was 40 years.

Seven of the hypocomplementaemic patients presented clinically with a nephrotic syndrome, one with an acute glomerulonephritis and two with asymptomatic proteinuria. There was no obvious difference in course or prognosis between patients with a normal or lowered B_{1A} level, but the number of patients is too small and the period of observation too short to allow definite conclusions to be drawn. The B_{1E} level was determined in eight patients and was found to be increased in two, normal in one and borderline in one. In only two patients was a slightly decreased value found. The B_{1E} level also showed little tendency to change during prolonged observation. It must be emphasized that no evidence for SLE could be found in any of the patients with MPGN.

Systemic lupus erythematosus

Renal biopsy was obtained from 10 of the 17 patients with SLE. Glomerulonephritis with diffuse proliferative changes was found in four patients (Fig. 1). Three of these patients had a nephrotic syndrome. The B_{1A} levels in these three patients were the lowest of the whole SLE group; the same tendency was evident for B_{1E}. The fourth patient with glomerulonephritis demonstrated a severe degree of sclerosis and hyalinization in the biopsy and had a severe renal insufficiency. The B_{1A} and B_{1E} levels were moderately decreased in this patient. One of the patients without glomerulonephritis in the biopsy had general manifestations such as fever, skin changes and muscular complaints. The B_{1A} level was 51% and the B_{1E} level 48%. Three patients with-

out abnormalities in the renal biopsy and with a quiescent clinical picture had normal B_{1A} and B_{1E} levels. One patient had a severe nephrotic syndrome without light microscopical changes in the biopsy Immunofluorescent investigations could unfortunately not be done. The B_{1A} level was normal, but the B_{1E} level was slightly lowered. Finally there was one patient in the group biopsied who had been treated by repeated haemodialyses for terminal renal insufficiency The biopsy showed severe vascular changes, with few signs of glomerulonephritis. The B_{1A} level was lowered, the B E level practically normal.

None of the seven non-biopsied patients had a nephrotic syndrome or any obvious signs of glomerulonephritis in the form of abnormal urinalysis, hypertension or disturbed renal function. At no time was the B_{1A} level of patients in this group decreased to the extent seen in patients with diffuse glomerulonephritis and a nephrotic syndrome. The B_{1E} level in these patients was lowered in three and normal in three individuals.

In only one of the 16 patients with SLE was the B_{1A} lowered and B_{1E} normal. This must be compared with MPGN where such relationship was regularly found.

The B_{1A} and B E levels were followed for a prolonged period in four patients (Fig. 2). Two of these patients (TD and WSC) were treated during this period with corticosteroids in large doses (60 mg prednisone/day). Both complement factors rose and did so simultaneously This is in contrast to the findings in APGN where the B_1 level tended to rise much sooner than the B_{1A} level.

DISCUSSION

The serum complement level in renal disease has mainly been investigated in children (6, 8 12, 18 19). *Some series include adult patients (1 7) but* do not distinguish between age groups. It is therefore of significance that in this study in a group of adult patients, the findings in children regarding a lowered B_{1A} level in APGN MPGN and SLE are confirmed.

The early decrease in B level in APGN and the characteristic gradual rise during the subsequent weeks are an important diagnostic aid in clinical situations. When the diagnosis of APGN is considered, throat cultures are often negative due to previous antibiotic therapy and a rise in ASO titre may not occur often a late manifestation and, moreover not specific for group A β-haemolytic streptococci. Although not encountered in this study the B_{1A} level may be normal in APGN If the first blood sample is taken too late the early decrease in B_{1A} level may be missed. In addition, proven cases of APGN ha e been described in whom the B_{1A} level was normal even at a very early stage of the disease (7 17)

The occasional finding of lowered B_{1E} levels in a very early phase of APGN is in accordance with the reports of Kohler and Ten Bensel (10) and Gewurz et al. (5) The B_{1E} level returns rapidly to normal, so that many cases can be differ entiated from SLE by a short follow-up examination. The rapid rise of B_{1E} in patients with APGN is in sharp contrast to the completely parallel rise of both factors which was observed during corticosteroid treatment of SLE. A similar course has been described by Gewurz et al. (5). It seems possible that the way in which the complement system is activated differs in these two diseases.

MPGN may present clinically as an acute nephritis. Determination of B_{1A} and B E levels in such patients will usually not distinguish them from APGN Follow-up examination for a period of weeks to months will usually bring out the difference. The B_{1A} level, however occasionally rises in MPGN and, on the other hand, adults with APGN may take up to 4 months to achieve normal B_{1A} levels.

From this in estigation it is clear that, in patients in whom a decreased B_{1A} level is found, quantitative determination of the serum B_{1E}-globulin can provide valuable information for the differential diagnosis between MPGN and SLE. With two exceptions, all MPGN patients with *low B levels had normal B level. In con*trast, all patients with SLE (with one exception) and lowered B levels had also low B level.

According to some authors (8) MPGN is a renal disease which only occurs in children and young adults I fact, in this study five of the seven hypocomplementaemic patients were young adults On the other hand three patients were 41 47 and 65 years old. This disease is therefore by no means confined to the youth.

A normal B_1 lev l was found in a number of

patients with MPGN However in comparison with the other categories of renal disease most of these patients had levels in the lower part of the normal range and no value higher than 107 % was encountered. No follow-up was made on these patients, so that the possibility of a lowered B_{1A} level at another stage in the disease process cannot be excluded. This has been observed by Cameron et al. (1) in a number of patients.

In the group of patients with MPGN investigated by us, no difference in age, sex, clinical presentation, course prognosis or extent of histological damage could be found between those with a normal and those with a decreased B_{1A} level. It therefore seems reasonable to follow the suggestion of Cameron et al. (1) and to define this group of patients with a primary renal disease not on the basis of a lowered B_{1A} level but on the histological picture.

The clinical diagnosis of SLE is often not easily made due to the multiplicity of symptoms. It is well known that the determination of B_{1A} levels can aid in the diagnosis (14). This was the case for example, with patient TD (Fig. 2) in whom the B_{1A} levels were low at a stage at which the antinuclear antibodies were not detectable. It has already been mentioned that, in all patients with SLE (with one exception) and lowered B_{1A} levels, also a low B_{1E} level was found. Moreover three of our patients with SLE had decreased B_{1E} levels with normal B_{1A} levels. It therefore seems possible that the determination of B_{1E} levels could form a useful screening test for SLE. The extent to which the level of B_{1E}-globulin is an indicator of the activity of the process, as seems to be the case for the B_{1A} level (7 17) is not clear from this investigation.

In summary from our findings the conclusion can be drawn that the determination of B_{1E}-globulin in addition to B_{1A}-globulin provides useful information for the distinction of different types of glomerulonephritis.

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THROMBOPHLEBITIS FOLLOWING INTRAVENOUS LIGNOCAINE INFUSION

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Abstract. A high incidence of thrombophlebitis has been noted in a Coronary Care Unit, where 2% lignocaine was given prophylactically to every second patient. The incidence of thrombophlebitis after 16–24 hours was about 50% hen giving glucose as drip in association with lignocaine from pump. When only glucose drip as given, or hen lignocaine was replaced by glucose in the pump the incidence was 4–10%. Therefore, lignocaine solution seems to promote the development of thrombophlebitis in i. infusions.

During the last decade lignocaine has in many centres become the drug of choice in the treatment of ventricular tachyarrhythmias occurring in association with acute myocardial infarction (AMI) (5 6 8–11). The reduction of mortality in Coronary Care Units (CCU) has been claimed to result largely from the prevention of malignant ventricular tachyarrhythmias following the use of lignocaine (7 9 11). In controlled studies the prophylactic use of lignocaine infusion at a rate of 2–2.5 mg/min has been shown to result in a significant reduction of ventricular tachyarrhythmias in AMI with minor side-effects (13 15). The safety and efficacy of i.v. lignocaine infusion at controlled rates ha e resulted in its widespread use in the elective management of ventricular tachyarrhythmias. Furthermore, it has been recommended prophylactically in AMI (15) although this approach has been disputed (14).

A high incidence of thrombophlebitis following lignocaine infusion was observed in our CCU and has to our knowledge not been reported earlier. This finding initiated the following study

MATERIAL AND METHODS

During the study period in the CCU at Serafimerlasarettet an i. 5.5 glucose infusion drip as started as soon as possible following each admission. The infusion set was connected to short polyethylene catheter (Infant-leny1® i.d. 1.45 mm, length 70 mm, Stille, Sweden), which was inserted percutaneously into a vein in the hand or arm. The procedure was performed in standardized way by specially instructed nurses.

During period group of randomly selected patients in addition received i. lignocaine from the admission. A single injection of 75 mg (3.75 ml) 2 solution lignocaine was given during 2 min and followed by infusion at constant rate of 2 mg/min, using an electric infusion pump (B Braun, Melsungen, West Germany). This group is called the glucose-lignocaine group. The pump was loaded with a 50 ml syringe filled ith lignocaine solution (Xylocain® Astra, Sweden) and connected to the 3-way tap on the catheter. The 50 ml syringe had to be exchanged every 8th hour. The remaining patients constitute control group, hic as given only the 5.5% glucose infusion drip, and is called the glucose group. During subsequent period second control group, in addition to the glucose drip also received 5.5% glucose from the pump, referred to as the glucose-glucose group. The same amount of fluid, about 1 000 ml in 24 hours, was given in the three groups.

The patients were examined every hour around the clock for signs of thrombophlebitic reactions. Thrombophlebitis was diagnosed when the area over the catheter end or the proximal part of the vein, was red and tender. The judgement as made by the nurses and recorded on special form, which also included information on any incidental paravascular administration. In addition, when the drip was stopped or pulled out by the patient, this was recorded and the patient was withdrawn from the study. Either incident happened in 10 patients. Usually the lignocaine and glucose infusions were continued throughout 24 hours, but were occasionally interrupted earlier. Therefore the comparison between the groups concerning the incidence of thrombophlebitis has been based upon an evaluation f each infusion made during successive 8-hour intervals for period up to 4 hours.

The material consisted of 113 patients, 30 (77) in the glucose-lignocaine group, 57 (46%) in the glucose group and 31 (77%) in the glucose-glucose group. Age and sex distribution and the incidence of verified AMI did not differ significantly between the groups.

Table I. *Incidence of thrombophlebitis during successive 8-hour intervals in the three trial groups*

	Interval (h)			
	0–8	8–16	16–24	
Glucose group				
Infusions (*n*)	52	49	45	
Thrombophlebites (*n*)	0	1	4	
(%)	0	2	9	
Glucose-lignocaine group				$p < 0.001$
Infusions (*n*)	30	29	25	
Thrombophlebites (*n*)	1	2	13	
(%)	3	7	52	
Glucose-glucose group				$p < 0.01$
Infusions (*n*)	31	29	23	
Thrombophlebites (*n*)	0	1	1	
(%)	0	3	4	

The remainder of the group comparisons were not statistically significant.

During the study 12 patients in the glucose-lignocaine group, 20 in the glucose group and 11 in the glucose-glucose group also received standard doses f frusemide, procainamide methyl scopolamine, ouabain, phenytoin or electrolyte solutions through the same catheter. The administration of any of these drugs was not overrepresented any of the trial groups related to the incidence f ophlebitis.

y the pH for the 5.5% glucose infusion fluid the mixture of glucose and lignocaine were measured.

RESULTS

The incidence of thrombophlebites, none of which was suppurative, is presented in Table I, where the three trial groups have been compared. The result of these comparisons favours a causal relationship between infusion of lignocaine solution and the development of thrombophlebitis.

The catheters were inserted at different locations as shown in Table II. There was no significant difference between the trial groups as regards the various locations, and no over representation for thrombophlebitis at any of the sites of insertion.

Repeated pH determinations showed levels between 4.25 and 4.40 for the 5.5% glucose solution. The pH for the appropriate mixtures of lignocaine and 5.5% glucose fell in the range of 6.25–6.40.

DISCUSSION

More than every second infusion in the glucose lignocaine group gave rise to a thrombophlebitis within 4 hours. A thrombophlebitis is a minor complication but may give the patient pain or discomfort for a few days and may increase the risk of bacteriaemia (1). None of the thrombophlebitic reactions in this series was, however suppurative.

It follows from the trial design and the results achieved that the lignocaine solution seems to be the sole factor behind the increased rate of thrombophlebitis.

The additives to the lignocaine solution, that is sodium chloride and methyl para-oxybenzoate, are not known to cause thrombophlebitis in these concentrations. Nor does the pH or osmolarity seem to be the responsible thrombophlebitic factor as the addition of lignocaine changes the pH of the glucose solution in the physiological direction, and changes the osmolarity very little. There is no support for any assumption that a change in the composition of the lignocaine solution would be beneficial.

Another possibility of reducing the incidence of thrombophlebitis would be to lower the lignocaine concentration. A larger amount of fluid

Table II. *Locations of cannulas in the three trial groups*

	Glucose-lignocaine group		Glucose group		Glucose-glucose group	
Location	No. of infusions	No. of thrombophlebites	No. of infusions	No. of thrombophlebites	No. of infusions	No. of thrombophlebites
Hand	11	4	13	2	7	0
Wrist	11	8	13	1	13	2
Forearm	5	3	15	2	6	0
Cubital	3	1	11	0	5	0
Total	30	16	52	5	31	2

could be used which however in AMI might increase the tendency to pulmonary oedema from left ventricular failure. Alternatively the dosage could be lowered but a slower administration rate, 0.5 and 1.0 mg/min, has not resulted in antiarrhythmic efficiency in a recent study (2). Thirdly a lower intravascular concentration might also be achieved using a larger vein.

Different routes of administering lignocaine have been tried (16) and this would be another way of avoiding thrombophlebitis. Oral lignocaine does not give therapeutic serum concentrations and causes considerable side-effects (3 4). I.m. administration of lignocaine has been reported to give promising prophylactic and therapeutic antiarrhythmic effects (12, 17) However whether repeated i.m. injections will trouble the patient less than thrombophlebitis is an open question. Similarly changing the infusion site regularly is also inconvenient. The finding of no increase in incidence of thrombophlebitis during the first 16 hours does not necessarily mean that this time would be the interval for changing catheters, as there may be some delay in the development of symptoms of thrombophlebitis.

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EVALUATION OF THE PRIMARY IMMUNE RESPONSE IN UREMIA AND AFTER EXTRACORPOREAL IRRADIATION OF THE BLOOD

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Abstract. The development of cellular and humoral hypersensitivity has been examined in seven uremic patients on chronic hemodialysis, following primary immunization with brucella bacteria. Both types of hypersensitivity were detected by the leucocyte migration test (LMT) and the agglutination reaction, respectively. The primary cellular response to an antigen, as measured by means of the LMT, varied with the serum creatinine level. Before dialysis, when the serum creatinine was high, the LMT was normal; after dialysis, when the serum creatinine was relatively low, the LMT was reduced. When estimations were performed shortly after dialysis treatment, i.e. at relatively low levels of serum creatinine, the seven uremic patients developed cellular as well as humoral hypersensitivity to the same extent as non-uremic controls. Three of the seven uremic patients received extracorporeal irradiation of the blood (ECIB) prior to immunization with brucella bacteria, whereas four were vaccinated without preceding ECIB. ECIB appeared neither to attenuate nor to abolish the primary immune response to brucella antigen, as measured by means of the LMT and the agglutination reactions.

Using brucella hypersensitivity as a model, Søborg and Bendixen (9, 11) showed that specific, antigen-induced inhibition of the migration of peripheral leucocytes is a good in vitro indicator of cellular hypersensitivity in man. Brucella hypersensitivity of the cellular type is regularly induced by infection or vaccination with brucella bacteria, whereas the induction of humoral hypersensitivity is more variable (2, 6).

The immunosuppressive effect of extracorporeal irradiation of the blood (ECIB) before kidney transplantation in the goat is demonstrated by a significant prolongation of graft survival (5). ECIB has been applied as a therapeutic measure in human kidney transplantation and is presumably effective (13), although the results are still under investigation. In vitro detection of cellular hypersensitivity by means of the leucocyte migration test (LMT) in brucella-vaccinated patients who have received ECIB may provide some new information on the effect of ECIB on the cell-mediated immune response. It has been suggested (4, 14) that the chronic uremic condition per se has an immunosuppressive effect. The present paper reports a study of the development of cellular and humoral hypersensitivity after primary immunization with brucella bacteria in seven uremic patients and the influence of ECIB prior to immunization in three of these patients.

MATERIAL AND METHODS

Patient material. Seven uremic patients undergoing chronic, intermittent hemodialysis treatment twice a week were studied. The patients were brucella-negative as defined by a leucocyte migration index (MI) above 0.78 (9) with an antigen concentration of 2.5 $\times 10^{8}$ brucella bacteria/ml culture medium. Intracutaneous reactions were not performed.

Four patients (group 1) were vaccinated with 10^{9} heat-killed brucella bacteria without preceding ECIB. Three patients (group 2) were vaccinated in the same way after the end of an ECIB series. Two of these patients received ECIB immediately before the vaccination. One patient terminated the ECIB 4 months before vaccination, but the blood lymphocyte level was still low, about 300/µl.

Technique of ECIB. ECIB was performed partly with a stationary ^{60}Co source (1), partly with a transportable ^{137}Cs source (12). The employed transit doses were 300, 360 and 360 rads and the total doses 43 000, 49 400 and 44 400 rads.

LMT was carried out as described in detail by Bendixen & Søborg (3). Leucocytes were recovered from heparinized blood from the arterial side of the Scribner shunt and washed three times in Hank's balanced salt solution. The cells were transferred to capillary tubes, which were subsequently placed in tissue culture chambers containing 1 ml of tissue culture medium (TC 199) with 10% serum. Migration cultures were made in series without antigen and in series containing 2.5 $\times 10^{8}$ heat-killed brucella abortus Bang bacteria/ml culture medium.

Table I *Leucocyte migration indices (MI), serum agglutinin titres (AT) and serum creatinine values following immunization on day 0*

		Days after immunization								
		0			9			14		
Pat. no	ECIB	Serum creatinine (mg/100 ml)	MI	AT	Serum creatinine (mg/100 ml)	MI	AT	Serum creatinine (mg/100 ml)	MI	AT
1	0	6.0	0.82	0	5 0	0.71	0	7.0	0.69	0
2	0	2.3	1 16	0	6.4	0 69	0	6.0	0.70	0
3	0	7.0	1.03	0	4.0	0.87	0	12.5	1 16	0
4	0	4.8	0 99	0	17.2	0.74	50	5.2	1.08	200
5	+	4 9	0.96	0	7 1	0.89	0	4.8	1.05	100
6	+	4.3	0.83	0	3.8	0.71	0	0.8[a]	0.75	0
7	+	3.4	0.95	0	3.8	0.70	25	10.0	1.09	50

The patient was transplanted with necro-kidney on day 10.

The 24-hour migration areas were measured by planimetry and the migration inhibition was calculated by dividing the mean area of antigen-containing cultures by the mean area of antigen-free cultures The MI is thus numerical indicator of the antigen-induced inhibition. The MI with another antigen (fetal kidney extract) served as a control

Brucella agglutination tests were performed at the State Serum Institute with serum dilutions from 1 5 to 1 20 and 1 25 to 1 64 000 The results are expressed by the reciprocal value of the highest, final serum dilution

RESULTS

Table I shows the MIs and the agglutinin titres of the seven patients during the first 14 days of the investigation. Three patients (nos. 1 2, 4) in group 1 who were vaccinated without prior ECIB and two patients (nos. 6, 7) in group 2, who were vaccinated after the end of an ECIB series, showed antigen-induced inhibition of migration at some time during the period of study The mean value and standard deviation of MIs on day 9 were 0.75 ± 0.08 in group 1 and 0.76 ± 0.10 in group 2, on day 14 they were 0.81 ± 0.18 in group 1 and 0.96 ± 0.18 in group 2. These differences were not statistically significant. For all the patients the mean value and standard deviation of MIs were 0.85 ± 0.18 which is not signifi-

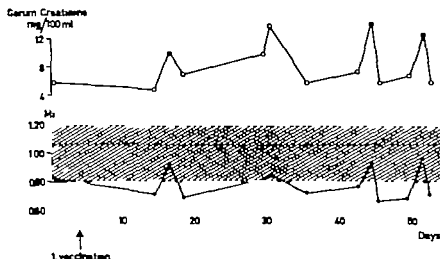


Fig 1 LMT in patient 1 after vaccination with 10⁹ heat-killed brucella bacteria. Serum creatinine values during hemodialysis treatment are indicated in the upper column.

cantly different from the value in non-uremic controls reported by Søborg (10).

Agglutinating antibodies were detectable in one patient in group 1 (no. 4) and in two patients in group 2 (nos. 5 7)

The MIs in one uremic patient on chronic hemodialysis treatment (no. 1) are shown in Fig. 1 It appears from the figure that the MI fluctuates considerably in parallel with fluctuations in serum creatinine. Before dialysis, when the serum creatinine is high, the MI is normal, after dialysis, when the serum creatinine is low the MI is reduced, indicating specific, antigen-induced migration inhibition.

DISCUSSION

The present study shows that uremic patients during chronic hemodialysis treatment may develop humoral as well as cellular hypersensitivity after brucella vaccination.

Furthermore, it shows that the uremic condition, here indicated by the serum creatinine level, inflicts some kind of damage on the leucocytes, with consequent impairment of their immunobiological functions. The factor(s) which injure the lymphocytes are apparently dialysable. The suppressive effect of uremia upon the immunological responsiveness is in accordance with the findings of Wilson et al. (14) and Bridges et al. (4).

Finally the *study shows that ECIB does not* attenuate or abolish the primary cellular response to an antigen, as measured by means of the LMT although the treatment causes a considerable reduction of the total number of lymphocytes in the organism (7 12). This in vitro finding is not unexpected, as it is known that the number of sensitized lymphocytes required for specific immunological information in migration inhibition reactions is comparatively small (8). In vivo, however considering the well established, immunosuppressive effect of ECIB in transplantation experiments (5) as well as the apparent effect in human transplantation (13) it appears that the efferent, cell-mediated immune reaction which damages the kidney graft is dependent upon the number of circulating sensitized lymphocytes.

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LYMPHOCYTE TRANSFORMATION TESTS BEFORE, DURING AND AFTER EXTRACORPOREAL IRRADIATION OF THE BLOOD

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Abstract. The lymphocyte responsiveness before, during and after extracorporeal irradiation of the blood (ECIB) has been investigated in 12 patients by means of lymphocyte transformation tests. Three kinds of stimulating agents were used: allogeneic cells in mixed cultures, tuberculin-purified protein derivative and phytohemagglutinin. Before ECIB the blast cell transformation of lymphocytes in uremic patients was identical to that of normal controls, per unit number of cells. The response per unit number of lymphocytes after stimulation with allogeneic cells and purified tuberculin was reduced significantly during ECIB but was normalized at the end of and after ECIB. The response after stimulation with phytohemagglutinin was unchanged, during as well as after ECIB. These results are compatible with the following hypothesis. The reduced immunological function of lymphocytes during ECIB is due to an accumulation of irradiation-damaged lymphocytes in the blood. After cessation of ECIB these partially damaged cells are cleared from the blood. The fraction of thymic-dependent, small lymphocytes left in the peripheral blood is therefore the same as before ECIB, although the total number is reduced.

Lymphocyte transformation can be defined as the morphological enlargement of small lymphocytes to larger lymphoblasts in vitro. This blast transformation can be measured quantitatively by assaying the total ^{14}C-thymidine uptake by a culture. The total DNA synthesis by the lymphocytes, determined in this way is a sensitive indicator of their transformation. The transformation can be effectuated by a variety of stimuli, such as specific antigens (e.g. transplantation antigens, tuberculin) antisera and non-specific mitogens (e.g. phytohemagglutinin (PHA))

Some experimental results indicate that only the small, thymic-dependent, recirculating lymphocytes (T-lymphocytes) are capable of blast transformation (3, 6, 13, 18). In man the evidence is less clear-cut and only indirect. Lymphocytes from patients with impaired delayed hypersensitivity or with thymic deficiency show a diminished response to different antigens and to PHA (7, 12, 15, 16, 17). Lymphocytes from patients with congenital agammaglobulinemia transform normally after antigen stimulation (2) and the response of lymphocytes in tuberculin-sensitized persons, after stimulation with tuberculin-purified protein derivative (PPD) is related to the degree of delayed sensitivity and unrelated to the level of circulating antibody (8)

The number of T-lymphocytes which respond initially to a specific antigen may be very small, while the fraction of T lymphocytes undergoing blast transformation by PHA is usually in the order of 90% (19).

In a previous study (23) it was demonstrated that the lymphocyte concentration in the peripheral blood changed in a characteristic way during prolonged extracorporeal irradiation of the blood (ECIB) After an initial rapid decrease in the lymphocyte concentration to about 30% of pretreatment value no further lymphopenia developed despite continued irradiation. Furthermore the lymphopenia remained unchanged during the first 8 months after cessation of ECIB

The in vitro lymphocyte transformation of this reduced lymphocyte population might provide some information about the relative quantity and immunological function of T-lymphocytes after ECIB

The purpose of this study was therefore to investigate the lymphocyte responsiveness before, during and after ECIB using three kinds of stimulating agents: allogeneic cells in mixed lympho-

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LYMPHOCYTE TRANSFORMATION TESTS BEFORE, DURING AND AFTER EXTRACORPOREAL IRRADIATION OF THE BLOOD

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Abstract. The lymphocyte responsiveness before, during and after extracorporeal irradiation of the blood (ECIB) has been investigated in 12 patients by means of lymphocyte transformation tests. Three kinds of stimulating agents were used, allogeneic cells in mixed cultures, tuberculin-purified protein derivative and phytohemagglutinin. Before ECIB the blast cell transformation of lymphocytes in uremic patients was identical to that of normal controls, per unit number of cells. The response per unit number of lymphocytes after stimulation with allogeneic cells and purified tuberculin was reduced significantly during ECIB but was normalized at the end of and after ECIB. The response after stimulation with phytohemagglutinin was unchanged, during as well as after ECIB. These results are compatible with the following hypothesis. The reduced immunological function of lymphocytes during ECIB is due to an accumulation of irradiation-damaged lymphocytes in the blood. After cessation of ECIB these partially damaged cells are cleared from the blood. The fraction of thymic-dependent, small lymphocytes left in the peripheral blood is therefore the same as before ECIB, although the total number is reduced.

Lymphocyte transformation can be defined as the morphological enlargement of small lymphocytes to larger lymphoblasts in vitro. This blast transformation can be measured quantitatively by assaying the total ^{14}C-thymidine uptake by a culture. The total DNA synthesis by the lymphocytes, determined in this way is a sensitive indicator of their transformation. The transformation can be effectuated by a variety of stimuli such as specific antigens (e.g. transplantation antigens, tuberculin) antisera and non-specific mitogens (e.g. phytohemagglutinin (PHA)).

Some experimental results indicate that only the small, thymic-dependent, recirculating lymphocytes (T-lymphocytes) are capable of blast transformation (3, 6, 13, 18). In man the evidence is less clear-cut and only indirect. Lymphocytes from patients with impaired delayed hypersensitivity or with thymic deficiency show a diminished response to different antigens and to PHA (7, 12, 15, 16, 17). Lymphocytes from patients with congenital agammaglobulinemia transform normally after antigen stimulation (2) and the response of lymphocytes in tuberculin-sensitized persons, after stimulation with tuberculin-purified protein derivative (PPD) is related to the degree of delayed sensitivity and unrelated to the level of circulating antibody (8).

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The in vitro lymphocyte transformation of this reduced lymphocyte population might provide some information about the relative quantity and immunological function of T-lymphocytes after ECIB.

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Table I *Results of ABO type and HL–A typing of the cell donors*

Pat. no.	Cell donor no.	Age (y)	Sex	ABO type	HL–A type LA	HL–A type Four
1		48	♂	O	2, 3	Fe 31/8 MK
	S_1 1	30	♂	A	1 3	LND CM
	S_1 2	21	♂	A	1 9	8, Sa533
2		29	♂	O	3 7	407*
	S_1 1	30	♂	A	1 3	LND CM
	S_1 2	21	♂	A	1 9	8, Sa 533
3		54	♂	A	2, Li	FJH SL–ET
	S_1 3	22	♂	O	10, 11	8, 4A2
	S_1 4	24	♂	A	1 Li	8 MaKI
4		45	♂	O	Li	7 FJH–AJ
	S_1 5	25	♂	O	2, Li	7
	S_1 6	24	♂	A	11 Li (Li–219)	R, MaKI
5		50	♂	A	2, 9	ET AAAJ
	S_1 7	22	♂	A	1 3	12, LND
	S_1 8	22	♂	A	9 Ba	12, 13
6		40	♀	O	2, 8	SL(ET)
	S_1 7	22	♂	A	1 3	12, LND
	S_1 8	22	♂	A	9 Ba	12, 13
7		51	♂	O	3 Li	8
	S_1 9	28	♂	A	9 Li	5
	S_1 10	28	♂	O	1 10	12, 8
8		23	♀	B	1	R, 12
	S_1 4	24	♂	A	1 Li	8 MaKI
	S_1 11	22	♂	O	9 Li	R, Fe31/8
9		25	♂	B	Li	R, MaKI
	S_1 7	22	♂	A	1 3	12, LND
	S_1 3	22	♂	O	10, 11	8, 4A2*
10		36	♀	O	Li	LND MaKI
	S_1 3	22	♂	O	10, 11	8 4A2*
	S_1 4	24	♂	A	1 Li	8, MaKI
11		47	♀	O	3, Li	R, BB
	S_1 2	21	♂	A	1 9	8 Sa533
12		53	♀	O	1 Ba	12, 5
	S_1 2	21	♂	A	1 9	8, Sa533

cyte cultures (MLC) PPD and PHA. In addition the effect of in vitro irradiation of normal lymphocytes was investigated by the same methods.

MATERIAL AND METHODS

The material consisted of 11 uremic patients on chronic dialysis treatment, who received ECIB prior to kidney transplantation, and one non-uremic patient who received ECIB before cornea transplantation (11). The age, sex, ABO type and tissue type of the 12 patients are shown in Table I.

Technique of ECIB. ECIB was performed partly with a transportable ^{137}Cs source, partly with a portable $^{90}Sr/^{90}Y$ source (23). The radiation doses were defined as follows: 1) Transit dose (TD) is the radiation dose received by any item while in transit through the radiation field. TD is proportional to the dose rate of the source and the volume of blood in the coil within the radiation field, and inversely proportional to the blood flow rate. 2) The number of blood volumes (BV) radiated is calculated from the measured blood flow rate through the irradiator and the patient's BV. 3) The total radiation dose is a product of TD and number of BV radiated.

ECIB of the patients. The average TD, number of BV radiated and total radiation dose for the 12 patients are presented in Tables II, III, IV together with the lymphocyte concentration in the peripheral blood. Four patients (nos. 1–4) received a mean TD of 330 rads, five (nos. 5–9) 100 rads and two (nos. 10 11) 19 rads. The non-uremic patient (no. 12) received a mean TD of 300 rads. Patients 1–9 and 12 were treated in a mobile gamma unit and ECIB was given 6–10 hours per day 3–4 days per week, in total about 100 hours. Patients 10 and 11 were treated in a new portable beta unit and ECIB was given continuously for about 150 hours.

Radiation in vitro of blood and lymphocytes was performed on five healthy volunteers. Three types of experiments were carried out: (*a*) the survival of lymphocytes in cultures after irradiation, (*b*) the blast cell transformation of lymphocytes after irradiation, and (*c*) ^{14}C-thymidine uptake after irradiation of lymphoblasts.

(*a*) The blood was led in a single pass from a cubital vein through an external blood tube past the radiation field of the ^{137}Cs unit. The blood flow rate was kept constant at 100 ml/min and a total amount of 200–300 ml was withdrawn. Three TDs were used, 100, 300 and 650 rads. Unirradiated blood passing through the tube at the same flow rate served as control. After irradiation the lymphocytes were isolated and cultured, without stimulating agents, as described below.

(*b*) The blood was treated in the same way as described under (*a*), but after isolation the lymphocytes were stimulated with allogeneic cells in MLC with PPD and PHA before the cultures were prepared.

(*c*) Lymphocytes from four normal persons were first isolated, stimulated with allogeneic cells, PPD and PHA and then cultured for 72 hours as described below. After culture for 72 hours the cells were diluted in 50 ml, 37°C isotonic NaCl and irradiated in the ^{137}Cs source with four TDs, 100, 300, 500 and 800 rads. Unirradiated, stimulated cells from the same experiment served as controls. After irradiation the cells were concentrated, incubated with ^{14}C-thymidine for 20 hours and harvested.

Lymphocytes. Peripheral blood lymphocytes were obtained from freshly drawn blood which was defibrinated by shaking with glass beads. The lymphocytes were isolated as described by Bøyum (1). Two ml of the defibrinated blood was mixed with an equal volume of medium (TC 199 Difco), layered on the top of 4 ml mixture of Isopaque-ficoll (density 1.077) and centrifuged for 20 min at 750 g. The layer of medium, which contains approximately 95% lymphocytes, was removed with a curved Pasteur pipette. These cells were washed three times in

Table II. *Data on four uremic patients treated with ECIB*

		ECIB				Lymphocyte transformation tests				
		Day	MCED[a] (rads)	Nos. BV radiated	Lymphocytes /µl blood	$R+S_1m$	$R+S_2m$	S_1+S_2m	R+PPD	R+PHA
Pat. 1	Before	−3				2 940	6 800	4 820	5 445	14 175
(mean TD		0			1 600	4 715	13 205	3 815	13 095	23 920
320 rads)		8	18 340	56	650	775	3 806	2 435	2 140	21 430
S,1 S,2	During	11	31 305	97	465	800	3 215	4 205	1 450	23 820
		14	44 735	136	605	3 100	5 090	7 440	6 605	22 920
		18	56 945	180	205	1 600	5 610	16 710	2 780	14 620
	After	+5	78 605	230		4 530	2 405	8 600		10 605
Pat. 2	Before	−3				2 310	5 185	4 820	5 730	22 010
(mean TD		0			1 680	5 095	7 110	3 815	6 015	23 875
295 rads)		4	19 130	65	580	3 255	5 710	6 745	4 755	24 518
S,1 S,2		8	27 550	94	610	1 670	3 860	2 435	2 320	20 040
	During	11	31 840	108	675	1 880	5 495	4 205	3 800	15 490
		14	38 800	136	340	3 970	5 805	7 440	5 445	20 310
	After	18	50 825	177	395	4 430	7 970	16 709	6 510	20 300
		+6	69 815	240		6 470	7 020	8 600	7 955	1 593
Pat. 3	Before	−3			1 065	760	1 195	2 245	2 945	17 145
(mean TD		0			475	1 620	1 550	2 115	8 170	18 575
300 rads)	During	15	37 800	124	110	3 375	4 575	3 265		13 870
S,3 S,4	After	+4	54 700	180		1 055	2 370	2 070		3 860
Pat. 4	Before	0			1 600	1 789	7 100	4 065	4 215	6 590
(mean TD										
415 rads)	During	4	26 500	65	175	1 655	2 345	3 945	1 340	14 847
S,5 S,6										

[a] Mean cumulative erythrocyte dose (total radiation dose).

medium and then resuspended in medium supplemented with 30% serum pool (obtained from the blood bank and stored at −80°C). The concentration of lymphocytes was adjusted to 10^6/ml. The suspensions were transferred in 500 µl aliquots to culture vials (white soda glass with screw caps). The cultures were maintained at 37°C in 5% CO_2-atmospheric air mixture for 92 hours without change of medium.

Stimulation / cultures. Reconstituted *PHA* (Wellcome), 15 µl/ml, or PPD (Statens Serum Institut, Copenhagen), 1 µl and 2 µl/ml, was added to the cultures. *Stimulating allogeneic cells ere treated with mitomycin C (Sigma),* 50 µl/ml, for 20 min at 37°C before washing. The concentration of stimulating lymphocytes as adjusted to 0.5 10^6 1.0 10^6 and 2.0 10^6/ml and mixed cultures were prepared using equal olumes of responding and stimulating suspensions. Unstimulated controls and mitomycin controls ere included. Furthermore day-to-day results ere compared with MLCs from the tw normal controls ho served as lymphocyte donors in each test performed on the ECIB-treated patients (Tables II, III, IV). The proliferative response was assayed 9 hours after preparation of the cultures.

After 20 hours' incubation with 50 µl ^{14}C-thymidine (sp. a. 60 µCi/mM, The Radiochemical Centre, Amersham, England), the cells were collected on glass fiber filter and processed for liquid scintillation counting as described by Sørensen et al. (21, 22) with the exception that incubation for 4 hours with Hyamine as omitted, because the results were found to be identical with and without Hyamine in repeated experiments. All samples ere counted in Packard Tri-Carb Liquid Scintillation Counter and all results were expressed as cpm/250 000 responding cells, after subtraction of the unstimulated culture. When stimulation with different antigen concentrations was used, results from the culture with the highest response were applied.

HL-A typing was undertaken by Drs Kissmeyer-Nielsen, Svejgård and Staub-Nielsen, and performed at the Tissue Typing Laboratory Rigshospitalet, Copenhagen, according *to the methods published elsewhere (10).*

Leucocyte and differential counts were performed daily

RESULTS

The match grades of the patients and their controls are presented in Table I There were one or more antigenic differences between the patients responding cells and the stimulating cells of the lymphocyte donors. The results of lymphocyte transformation tests in the 12 patients before, during and after ECIB are shown in Tables II, III and IV together with the ECIB data and the lymphocyte concentration in the blood The results of repeated *MLC tests between normal*

Table III. *Data on five uremic patients treated with ECIB*

		ECIB Day	MCED[a] (rads)	Nos. BV radiated	Lymphocytes /µl blood	Lymphocyte transformation tests R + S_1m	R + S_2m	S_1 + S_2m	R + PPD	R + PHA
Pat. 5	Before	0			1 985	3 975	2 905	2 090	3 815	10 365
(mean TD		13	7 880	79	570	3 875	820	2 145	5 875	23 970
100 rads)	During	20	14 140	141	495	2 675	550	3 055	2 145	17 660
$S_1$7 $S_2$8		24	17 100	168	130	1 090	150	2 070		15 460
	After	+4	18 140	178	350	3 390	1 970	1 965		18 305
Pat. 6	Before	−1			1 050	4 425	1 956	1 807	10 390	28 915
(mean TD		0			1 120	2 815	1 140	2 090	7 590	11 895
100 rads)		13	15 830	158	490	1 406	660	2 145	3 650	24 285
$S_1$7 $S_2$8	During	20	25 520	230	385	810	850	3 055	3 620	23 420
	After	24	29 680	272	290	475	185	2 070		16 815
		+4	31 500	292		4 610	3 155	1 915		16 570
Pat. 7	Before	0			1 670	11 800	8 130	5 920	7 157	25 410
(mean TD		2	3 490	35	840	1 065	890	4 786	175	15 115
100 rads)	During	6	6 320	63		2 530	2 070	1 497	1 990	14 080
$S_1$9 $S_2$10		30	19 830	197	215	3 445	2 015	2 040	4 420	21 570
	After	+7	21 900	217		3 285	2 635	4 370	1 665	22 330
Pat. 8	Before	−3				3 230	3 570	2 235	1 850	
(mean TD		0			2 080	2 135	1 730	6 805		
100 rads)	During	7	6 540	65	285	1 280	1 340	1 645		
$S_1$4 $S_2$11	After	+15	15 015	149	325	2 575	2 130	3 440	2 455	
Pat. 9	Before	−3			1 845	9 566	7 705	4 795	9 005	14 380
(mean TD	During	5	9 860	95	545			2 170	3 615	12 920
110 rads)										
$S_1$7 $S_2$3										

[a] Mean cumulative erythrocyte dose (total radiation dose).

Table IV. *Data on two uremic and one non-uremic patient treated with ECIB*

		ECIB Day	MCED[a] (rads)	Nos. BV radiated	Lymphocytes /µl blood	Lymphocyte transformation tests R + S_1m	R + S_2m	S_1 + S_2m	R + PPD	R + PHA
Pat. 10	Before	0			1 330	4 640	2 445	3 720	4 790	27 045
(mean TD		4	4 700	243	1 150	1 610	915	2 245	1 010	15 025
19 rads)	During	7	8 035	357	525	2 275	765	2 115	3 990	22 910
$S_1$3 $S_2$4	After	+11	9 090	475	490	3 875	2 370	3 265	2 495	18 240
		18	9 090	475	445	1 480	1 805	2 070		23 145
Pat. 11	During	1	1 220	64	2 170	6 315				
(mean TD		3	3 700	194	1 475	9 250				
19 rads)		5	6 100	321	510	7 265				
$S_1$2	After	+2	8 670	454	740	5 130				
		+3	8 670	454	440	6 275				
Pat. 12	Before	−1			1 220	9 850				
(mean TD		1	4 320	14	780	7 690				
300 rads)	During	3	16 560	55	290	5 085				
$S_1$2		8	36 840	123	125	5 290				
		9	42 450	142	295	5 425				

[a] Mean cumulative erythrocyte dose (total radiation dose).

controls are also presented, the day-to-day variation was 40 %.

Lymphocyte transformation test before ECIB. The mean value and S.D. of ¹⁴C-thymidine incorporation (cpm/250 000 cells) after stimulation in MLC, with PPD and PHA in ten uremic patients (nos. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10) before ECIB were: MLC 4 849 ± 3 197, PPD 6 043 ± 2 500, PHA 18 227 ± 6 793. In 9 normal controls the results were MLC 3 780 ± 1 492, PPD 3 586 ± 2 515, PHA 20 125 ± 2 334. Using the *t*-test the results from uremic patients were not significantly different from the results from normal persons ($p > 0.1$, $0.05 > p > 0.01$, $p > 0.1$).

Lymphocyte transformation during ECIB. The mean value of ¹⁴C-thymidine incorporation (cpm/250 000 cells) after stimulation with the three kinds of agents, before and during ECIB is presented in Fig. 1. A significant reduction in the MLC and PPD response during ECIB was found ($p < 0.01$). In contradistinction the PHA response remained unchanged ($p > 0.1$). The change in MLC, PPD and PHA response, expressed in % of the value obtained before ECIB is shown in relation to the total radiation dose in Fig. 2. A pronounced reduction (40 % and 50 %), per unit number of cells, in the MLC and PPD response was found in the beginning of ECIB where the lymphocyte concentration was still decreasing. Later during ECIB, when the lymphocyte concentration was constantly reduced to about 30 % of pretreatment value the MLC and PPD response per unit number of cells was increasing again. The PHA response was

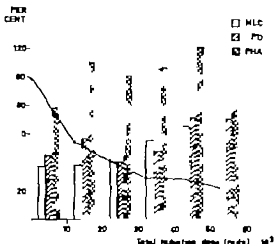


Fig. 2. Lymphocyte transformation test after stimulation with allogeneic cells in MLC, PPD and PHA during ECIB. Abscissa: Total radiation dose (rads). Ordinate: ¹⁴C-thymidine incorporation (cpm 250 000 cells) in % of pretreatment values. The solid line represents the mean lymphocyte concentration in % of pretreatment value.

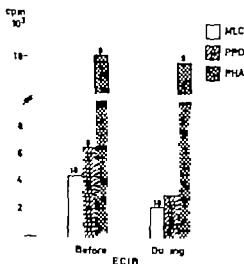


Fig. 1. Lymphocyte transformation test after stimulation with allogeneic cells in MLC, PPD and PHA before and during ECIB. Ordinate: ¹⁴C-thymidine incorporation (cpm/250 000 cells). The number of observations is shown above each column.

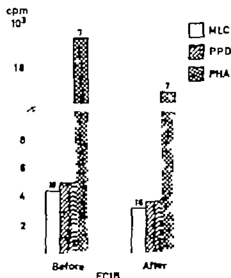


Fig. 3. Lymphocyte transformation test after stimulation with allogeneic cells in MLC, PPD and PHA before and after ECIB. Ordinate: ¹⁴C-thymidine incorporation (cpm/250 000 cells). The number of observations is shown above each column.

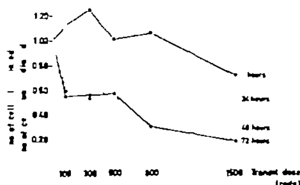


Fig 4 Irradiation-induced change in the lymphocyte concentration in cultures. Ordinate: number of lymphocytes irradiated. The different curves indicate lymphocyte counts at variable intervals after preparation of the cultures.

unchanged. If the lymphocyte count is multiplied by the ^{14}C-thymidine incorporation, the following results are obtained. The number of cells which respond initially to stimulation with allogeneic cells and PPD was constantly reduced during ECIB while the number of cells responding in PHA cultures decreased in parallel with the decreasing lymphocyte concentration.

Lymphocyte transformation tests after ECIB The mean value before and after ECIB of ^{14}C-thymidine incorporation (cpm/250 000 cells) after stimulation in MLC, with PPD and PHA is presented in Fig. 3 If the mean value and S.D are compared before and after ECIB in patients for whom both results are available no significant reduction is found in MLC, PPD and PHA cultures ($0.02 > p > 0.01$ $p > 0.1$ $p > 0.1$).

Radiation in vitro of blood and of lymphocytes If irradiated lymphocytes are cultures without stimulating agents (Fig. 4) a reduction to about 50% occurs within the first 24 hours after the culture is prepared. The results are corrected for spontaneous cell death of unirradiated cells.

The influence of increasing X-ray doses on blast transformation of normal lymphocytes after stimulation with allogeneic cells in MLC, with PPD and PHA is shown in Fig. 5 The results are expressed in % of values in the unirradiated cultures. With increasing X-ray doses the response in MLC and PPD cultures decreases. The coefficient of correlation was significant ($= -0.828$ $p < 0.001$ $r = -0.811$ $p < 0.001$). The responses after stimulation with PHA were unchanged ($r = -0.251$ $p > 0.5$) The irradiation-induced

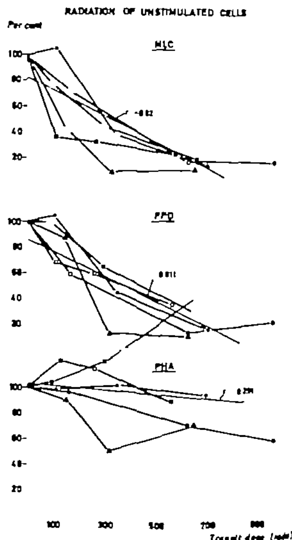


Fig. 5 Irradiation-induced change in blast transformation of normal lymphocytes after stimulation with allogeneic cells in MLC, PPD and phytohemagglutinin PHA. Ordinate: ^{14}C-thymidine incorporation (cpm/250 000 cells) in % of pretreatment values. Each symbol represents a lymphocyte donor – coefficient of correlation.

change in MLC and PPD was well correlated (Fig. 6) ($r = 0.878$ $p < 0.001$) whereas the change in MLC and PHA was not correlated ($r = 0.226$, $p > 0.5$)

The effect of irradiation in vitro on blast cells was comparable to the effect of irradiation in vitro on normal, non-transformed cells (Figs. 8 and 9).

DISCUSSION

It has been demonstrated that the immunological competence may become depressed in uremia (9

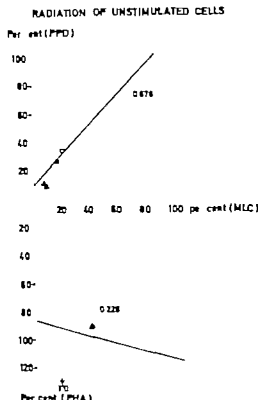


Fig. 6. Correlation between the irradiation-induced change in blast transformation of normal lymphocytes stimulated with allogeneic cells in MLC, PPD and PHA.

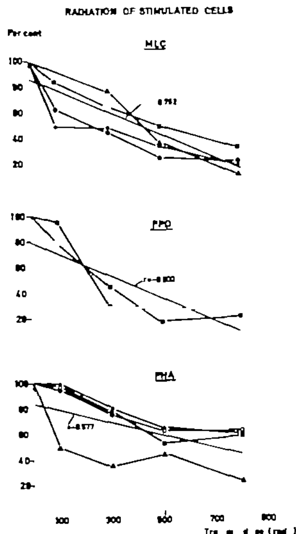


Fig. 7. Irradiation-induced change in ^{14}C-thymidine uptake of lymphoblasts. Ordinate: C^{14}-thymidine incorporation (cpm/250 000 cells) in % of pretreatment values. The blastogenic agents were allogeneic cells in MLC, PPD and PHA. Each symbol represents lymphocyte donor, – coefficient of correlation.

25). In a previous study (24) it was reported that the cellular hypersensitivity to brucella antigen in an uremic patient (as measured by the leucocyte migration test, LMT) was correlated to the serum creatinine concentration. The well dialyzed brucella-vaccinated patient shows inhibition in LMT to the same extent as normal controls, whereas the inhibition is diminished or disappears just prior to dialysis when the degree of azotemia is pronounced. The present study shows that the lymphocytes of well treated uremic patients on chronic intermittent dialysis may react with blast cell transformation after stimulation with allogenic cells in MLC with PPD and PHA to the same extent as normal lymphocytes. The well dialyzed but still uremic patient seems to be normally immunocompetent. The suppression of immunological competence in uremia (9, 25) might be caused by a higher degree of uremia than in the present material.

During and after ECIB the responsiveness of the lymphocytes after stimulation with allogenic cells in MLC and with PPD was first reduced and then normalized (Figs. 1, 2, 3) indicating that the fraction of thymic-derived lymphocytes (T lymhocytes) left in the peripheral blood was reduced during ECIB but becomes normal after ECIB. It is shown in Fig. 4 in this study and by Ewans and Norman (4) that 50% of irradiated, normal lymphocytes die within the first 24 hours when cultured in vitro. The reduced responsiveness per unit number of cells in MLC and PPD

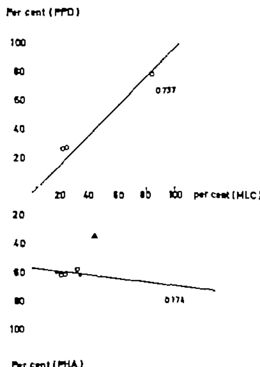


Fig. 8 Correlation between the irradiation-induced change in ^{14}C-thymidine uptake of lymphoblasts, triggered to blast transformation with allogeneic cells in MLC, PPD and PHA.

ight therefore be caused by an accumulation of irradiation-damaged lymphocytes in the blood during ECIB These cells are cleared from the blood after cessation of ECIB (5)

Considering the radiation-induced 50 % reduction of the number of lymphocytes in in vitro cultures (Fig. 4) it is surprising that the PHA response was unchanged in patients during as well as after ECIB and also unchanged after irradiation in vitro of blood or lymphocytes (Fig. 5) (14) As indicated by Schrek and Stefani (20) the explanation could be that PHA protects lymphocytes in vitro against radiation whether it is added 5 days before or even 2 days *after* irradiation. The effect was explained by radioresistance of the transformed lymphoblasts. This theory is in accordance with the results in this study which show an unchanged ^{14}C-thymidine uptake of irradiated lymphoblast in the PHA cultures (Fig. 7)

The effect of in vitro irradiation on the responsiveness of normal lymphocytes after stimulation with allogeneic cells and PPD (Figs. 5 and 6) can simply be explained by death of the irradiated cells when cultured. The number of lymphocytes left in the cultures, which are capable of blast transformation, after stimulation is halved. These specific antigens (transplantation antigens and tuberculin) therefore do not appear to provide protection of lymphocytes against radiation. The decrease in the ^{14}C-thymidine uptake of irradiated lymphoblasts shown in Fig. 7 supports this theory

It can be concluded that ECIB causes an effective and long lasting lymphopenia (23) but that the remaining lymphocyte population contains an unchanged fraction of immunologically competent T-lymphocytes.

ACKNOWLEDGEMENTS

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GAMMA HEAVY CHAIN DISEASE

Reports of Three Patients

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Abstract. Three cases of γ-heavy chain disease (γ-HCD) are reported. T of the patients had clinical picture of malignant lymphoma, similar to most of the cases earlier described. One patient, however, had benign process characterized by symptomless splenomegaly and moderate plasmocytosis in bone marrow and spleen. This patient, followed since 1964, is still alive and in good health. In all three cases the existence in serum and urine of an M-component with IgG specificity was demonstrated. Gel filtration and immunoelectrophoresis showed that these proteins had molecular weight below that of normal IgG and that they did not react with antisera against light chains. The clinical and morphological features of the earlier reported cases of γ-HCD are briefly reviewed and compared with the findings in our patients. It is further discussed whether the presence of an M-component consisting only of γ-heavy chains is an accidental finding in cases with proliferation of immunocompetent cells or whether patients with this abnormality represent uniform disease entity.

In 1964 five cases of a new syndrome heavy chain disease (γ-HCD) distinguished by a disturbance in the immunoglobulin production and a clinical picture of malignant lymphoma, were published (6, 20). The characteristic immunoglobulin abnormality consisted of the appearance in both serum and urine of a fragment of the IgG molecule closely similar to the Fc portion of the γ-chain, without evidence of a corresponding increase in the production of light chains. Since then six other cases have been reported in the literature (3, 12, 26, 30, 31). We report here three further patients in whom the immunological findings were consistent with a diagnosis of γ-HCD, one of them having a reticulum cell sarcoma, one a malignant lymphoma resembling Hodgkin's sarcoma and one a plasmocytosis with an apparently benign course.

Presented in part at the meeting of the Swedish Society of Hematology, April, 1970

CASE REPORTS

Case 1

A 75-year-old woman was admitted to Sahlgren Hospital, Göteborg in July 1969 because of lymphadenopathy, high fever and respiratory distress.

The patient's father and one brother had bronchial asthma. Since adolescence she had been troubled by rhinitis and bronchitis, but until 1960 she was otherwise in good health. Later on she showed signs of slight cardiac insufficiency and moderate hypertension. I 1965 she was referred to hospital because f signs of increasing cardiac failure. At examination generalized oedema, large pleural effusions and on X-ray an increase of the heart silhouette ere found. Her arterial oxygen saturation and ventilatory capacity ere reduced and he as considered to have combination of cardiac and respiratory failure. On treatment her condition rapidly improved. A marked eosinophilia of the peripheral blood and total WBC count of 10 000 mm³ ere noted. The bone marrow smear also showed an eosinophilia but as unremarkable in other respects. Six months later she returned with large unilateral pleural effusion ithout manifestations of cardiac failure. Cultures and cytological examinations of pleural fluid were negative. One year later she developed difficulties in alking and diagnosis of peripheral neuropathy as made at neurological examination. The marked eosinophilia of the peripheral blood and of the bone marrow was confirmed at repeated examinations during the following years. A slight but inconstant proteinuria as noted. Serum electrophoresis and ESR were normal.

In April 1968 the patient was reexamined because of dysphagia f some months duration. Over the thyroid isthmus and extending dow into the jugulum 3 6 cm firm, smooth, non-tender tumour was found. On both sides of this swelling conglomerates of enlarged lymph

nodes were observed. The patient was euthyroid. A fine-needle biopsy and a larger surgical biopsy specimen from the thyroid showed a marked infiltration of lymphocytes and plasma cells. Repeated tests for thyroid antibodies were negative. Paper serum electrophoresis showed a slight increase in the β-globulin fraction. Immunoelectrophoretic analysis revealed a distinct M-component with supposed IgG specificity. On the assumption that the patient had a chronic lymphoid thyroiditis she was treated with prednisolone and l-thyroxine and responded with a decrease of the thyroid mass and the lymph node swellings. The number of eosinophils both in the peripheral blood and in bone marrow were promptly reduced to normal values. When the dose of prednisolone later was decreased, the thyroid and lymphonodular swellings reappeared.

In July 1969 the patient's condition rapidly deteriorated with increasing dyspnoea and high fever. She was admitted to the hospital and at examination found to be dyspnoeic, febrile, tired, with slight oedematous swelling of both legs. In the left supraclavicular fossa and in the left axilla conglomerates of up to 1–2 cm soft, freely movable lymph nodes, and in the right supraclavicular fossa 2–4 cm lymph node of the same consistency were found. She had a moderate hepatomegaly and the tip of the spleen was palpable.

A chest X-ray revealed thickening of the pleural capsule over the right lung and small bilateral pleural effusions. Laboratory studies showed: Hb 11.7 g/100 ml, WBC 7 300/mm³ with a percentage of [illegible] stabs, 64 segmented neutrophils, 4 monocytes and 30 lymphocytes, platelets 120 000/mm³ and reticulocytes 2.0%. ESR 4 mm/h. Serum electrophoresis showed that the β-globulin fraction had increased to 1.4 g/100 ml and had the character of an M-component. Immunoelectrophoretic analysis showed the M-component to have IgG specificity but tests with antisera against light chains were negative. In concentrated urine an M-component of the same mobility was observed. A bone marrow aspiration revealed a slight increase of eosinophils, but no increase of plasma cells and no changes in the lymphocytic series. A skeletal X-ray survey was normal. Biopsy of a supraclavicular lymph node showed a picture most consistent with a diagnosis of reticulum cell sarcoma. Treatment with high doses of prednisolone was initiated resulting in a certain improvement in her general condition and a normalization of temperature. Irradiation was given against one supraclavicular and one axillar field. Two months later her condition improved so that she could be sent home, but she rapidly developed a leukopenia and had to be readmitted in bad condition with high spiking fever. Again she responded to steroids and, when the leukocyte counts normalized, a course of cyclophosphamide was instituted, causing a further improvement and a decrease of the glandular swellings.

The last two months of the patient's life were characterized by a progressive emaciation and periods of high septic fever, but no visible progress of her lymphadenopathy. 1 Nov she developed small haemorrhagic pustules under both mammae which later spread to a more generalized pyoderma. Blood cultures were consistently negative, but later Pseudomonas aeruginosa was cultured from the pustules. Treatment with antibiotics was only temporarily successful and she died in Jan. 1970.

Case 2

A 47-year-old woman was admitted to the County Hospital in Halmstad in Nov. 1969 because of fever and generalized lymphadenopathy of one week's duration.

In 1955 she had had rubeola and, some time after this, symptoms of dysphagia, dysarthria and weakness of the arm muscles appeared. Myasthenia gravis was diagnosed in 1957. In the following years she was treated with neostigmine. In 1962 she received a total irradiation dose of 4 400 rads against the upper mediastinum without definite evidence of a thymoma. After this a marked amelioration of her myasthenic symptoms was noted.

In July 1968 the patient was examined because of fatigue of some months duration and repeated upper respiratory tract infections. A slight leukocytosis was observed but the Hb value, the differential count, a sternal marrow examination, as well as the physical examination, were normal. One year later she was again referred for examination, now because of tenderness and swelling in the left parotid region. The examination, however, did not reveal anything abnormal. The following months were characterized by increasing fatigue, anorexia and weight loss. At the beginning of Nov. she rapidly developed high fever, profuse nightly sweating and increasing generalized lymph node enlargement.

Physical examination at the time of admission in Nov 1969 revealed a febrile and tired woman with conglomerates of 2–4 cm, rubbery slightly tender lymph nodes along both sternocleidomastoid muscles and in the cervical, submandibular and supraclavicular areas, giving the appearance of a "bull neck". The axillar and inguinal lymph nodes were enlarged to almost the same degree, making up firm and almost non-tender conglomerates. The tonsils were moderately swollen and the pharyngeal mucous membranes hyperaemic. The liver was palpable 4 cm beneath the costal margin. The lungs were clear and other physical findings were negative. The laboratory values were: Hb 11.0 g/100 ml, WBC 8 200/mm³, platelets 163 000/mm³ and reticulocytes 1.4%. The differential count showed a percentage of 23 stabs, 26 segmented neutrophils, 3 eosinophils, 1 basophil, 2 monocytes and 43 lymphatic cells, many of which had a plasmocytoid appearance. ESR 30 mm/h. She had a slight but inconstant proteinuria. A routine serum paper electrophoresis showed a moderate elevation of α-2 globulins and no definite M-component. Chest X-ray revealed bilateral parenchymal infiltrations and small pleural effusions. The hilar lymph nodes were not enlarged. A slight splenomegaly was observed on a plain X-ray. A skeletal X-ray survey was negative. A bone marrow aspirate showed an increased amount of lymphocytoid cells. Cells of the same type were observed in a fine-needle biopsy from the liver. Surgical biopsies were taken from two lymph nodes and the left tonsil, showing a diffuse infiltration of reticulum cells and plasmocytoid cells. A diagnosis of Hodgkin's disease was considered probable on basis of the pathological findings. The pulmonary infiltrates disappeared but the lymph nodes

increased further in size and she remained subfebrile. The peripheral blood contained up to 10% plasmocytoid cells. One month later the patient's condition rapidly deteriorated. The tonsils and the soft palate became swollen and she experienced considerable difficulties in swallowing. Palliative irradiation of this region was instituted with prompt effect.

The patient was referred to our department in the middle of Dec. 1969. At examination the lymph nodes in the neck, axillae and inguinal regions were up to 3–4 cm, conglomerated, firm, non-tender and freely movable in relation to the underlying tissues. The tonsils were swollen and hyperaemic. The liver was palpable 6 cm and the spleen 2 cm beneath the costal margin. Laboratory tests showed approximately the same values as reported above. Paper electrophoresis of serum revealed faint M-component in the β-region. Immunoelectrophoresis confirmed the presence of an M-component with IgG specificity but without reaction with antisera against kappa and lambda chains. A similar M-component was found on immunoelectrophoresis of concentrated urine. Treatment was initiated with combination of cyclophosphamide, vincristine and prednisolone, given in courses with an interval of two weeks. The patient's symptoms were promptly relieved during the following weeks. The lymph node swellings and the splenomegaly decreased and she became afebrile. The cytostatic treatment was continued for the next two months. The decrease of the lymph node swellings and the hepatosplenomegaly continued and her general condition steadily improved.

In March 1970 high septic fever reappeared together with bilateral parotid swelling. Chest X-ray showed bilateral small parenchymal infiltrates. She had moderate anaemia and WBC of 68 000/mm³ with 10–20% atypical cells of lymphoid or plasma cell nature. A new course of treatment with an increased dose of cyclophosphamide once more produced normalization of body temperature, clearing of the X-ray picture, decrease of the parotid swelling and return of WBC to almost normal values. This period of improvement, however, only lasted for a few weeks, whereafter all symptoms reappeared. The condition during the last month of the patient's life was characterized by high septic fever, emaciation, oedema, gastrointestinal bleeding, ascites, oral ulcerations, urinary infection and pancytopenia. Chest X-ray showed parenchymal infiltrations in both lungs. The marrow was found to be hypocellular with areas of necrosis and degeneration, for which reason the cytostatic treatment had to be abandoned. Blood cultures were negative and the fever unresponsive to high doses of antibiotics and steroids.

Case 3

A 70-year-old woman was in Nov. 1970 reexamined by us because of repeated upper respiratory tract infections.

The patient was in 1955 treated for cystopyelitis and in 1960 cholecystectomized, but was otherwise in good health up to 1963. On some occasions her ESR had been found to be moderately elevated. In Dec. 1964 she was hospitalized because of high fever, arthralgia and symptoms of cystopyelitis. On the day before she had been ordered a sulfa drug and developed an intense papulovesicular rash. At the time of admission she was in bad condition with serum creatinine 5.9 mg/100 ml and Hb 8.9 g/100 ml. From the urine a heavy culture of E. coli was obtained. After institution of tetracyclines she rapidly improved. The physical examination revealed moderate hepatosplenomegaly but no enlarged lymph nodes. In the face the allergic reaction had the character of discoid lupus erythematosus. The microscopic examination of a later performed punch biopsy was considered to be consistent with this diagnosis. Laboratory studies showed, besides the anaemia, WBC 3 600 mm³ with 40% lymphocytes and an occasional plasma cell in the differential count, platelets 260 000 mm³, reticulocytes 2.0%. ESR was initially very high, but later stabilized at a level of about 50 mm/h. The paper electrophoresis revealed a faint M-component between the β- and γ-regions. Only traces of protein were present in the urine. No LE cell phenomenon could be detected in several preparations. A chest X-ray revealed initially small bronchopneumonic infiltrates and basal atelectases in both lungs, which cleared up during the treatment. A skeletal X-ray survey was negative. A moderate plasmocytosis was noted in a bone marrow smear and the same finding was observed in smears from a fine-needle biopsy of the enlarged spleen.

During the following two years the patient was in good health. In July 1966 she had a left-sided lobar pneumonia, which responded promptly to treatment with penicillin. Her hepatosplenomegaly was of the same size as two years before. ESR 44 mm/h. In the paper electrophoresis the same M-component of slow β-mobility was noted. Immunoelectrophoretic analysis showed that both serum and concentrated urine contained an M-component with IgG specificity. No tests with antisera against light chains were performed at that time. The Bence Jones test of the urine was negative. A bone marrow aspiration showed 8% plasma cells. A skeletal X-ray survey revealed an osteoporosis, most marked in the thoracal and lumbar vertebrae, and a slight compression fracture of vertebrae Th XI. No punched-out areas or other localized destructions were seen. Soon after the hospital stay the patient went back to work and she has afterwards been in good health. During 1967 and 1968 she visited the outpatient department on three occasions. On these visits she had no anaemia and a normal ESR.

During the last months of 1970 she experienced repeated upper respiratory infections which cleared up. She felt slightly tired, and because of this she visited the outpatient clinic in Dec. for a general check-up. At the physical examination the liver was palpable 3 cm and the spleen 4 cm below the costal margin. No lymph nodes could be palpated. There was a very slight erythema over both cheeks. Her laboratory values were: Hb 11.0 g/100 ml, WBC 4 600 mm³ with percentage of 56 segmented neutrophils, 1 eosinophil, 2 monocytes and 41 lymphocytes, platelets 125 000 mm³ and reticulocytes 1.0%. A sternal bone marrow smear showed 11% well differentiated plasma cells. The serum M-component was this time subjected to a more detailed analysis and found to be a HCD protein of IgG specificity.

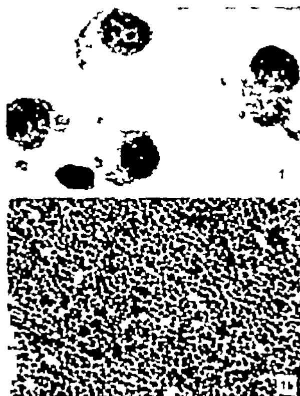


Fig. 1. Case 1. Characteristic morphological features. (*a*) Plasma cells in smear from fine-needle biopsy of thyroid tumour. Giemsa stain ×1 394. (*b*) Section of lymph node removed by biopsy. The normal architecture is replaced by a dense infiltration of uniform lymphoreticular cells. Van Gieson, ×175.

Morphological Findings

Case 1

Bone marrow. Smears of bone marrow were examined repeatedly from 1965 to 1969. A varying degree of eosinophilia (29–42%) was noted before steroid therapy was initiated. The number and appearance of lymphocytes and plasma cells were not abnormal.

Thyroid. Smears from a fine-needle biopsy in June 1968 revealed very little of thyroid follicular epithelium and a predominance of plasma cells, showing different degrees of maturity but as a rule well differentiated (Fig. 1*a*). Some intermediate forms between plasma cells and lymphocytes were observed. The puncture also contained some small mature lymphocytes, reticulum cells and eosinophilic leukocytes. A surgical biopsy from the thyroid isthmus, taken at the same time, showed only some small atropic remnants of colloid-containing follicles. Most of the specimen had the character of dense and very cellular lymphoreticular tissue. The lymphoid cells had a varying degree of maturity and were intermingled with plasma cells. The dominating cells were, however, large lymphoid cells, most of lymphoblastic appearance, and plasma cells. Characteristic reticulum cells with large nuclei were few in number as were neutrophilic and eosinophilic leukocytes.

Lymph node. Biopsy of a lymph node, from the supraclavicular area, made in June 1968 showed the normal lymph node architecture to be replaced by a very cellular lymphoreticular tissue dominated by lymphoblast-like cells and plasma cells of the same uniform appearance as in the biopsy from the thyroid gland (Fig. 1*b*). The lymphoid cells infiltrated the lymph node capsule and the surrounding fat tissue. Another lymph node excised one year later showed an identical microscopical picture. The morphological picture was considered consistent with a diagnosis of malignant lymphoma.

Autopsy findings. The post-mortem examination revealed a generalized lymphadenopathy with enlarged nodes around the thyroid gland, in the mediastinum, along the abdominal aorta and in axillar and supraclavicular areas. The thyroid gland was enlarged, and the tissue appeared homogenous, firm and pale. Spleen and liver were of normal size. The lungs contained some purulent bronchopneumonial infiltrates and a generalized oedema.

Microscopically no detailed examination was possible because of advanced postmortal autolysis. However, the same type of lymphoreticular cell proliferation, as had earlier been seen in the biopsies, could be identified in most of the lymph nodes and in the thyroid tissue. The liver showed a pronounced steatosis. Signs of lymphoid infiltration were seen neither in the liver nor in the spleen.

Case 2

Bone marrow. Aspirations of sternal marrow were performed four times between Nov. 1969 and May 1970. Atypical cells in a varying amount (2–10%) were observed. These cells had a lymphoid character with a coarse nuclear chromatin structure and some of them contained a nucleolus. However the cytoplasm was often more abundant, foamy and strongly basophilic with a small perinuclear zone. Some cells had definitely an appearance reminiscent of plasma cells. No increase of the eosinophilic leukocytes was observed.

Liver and spleen. In smears from a fine-needle biopsy from the liver basophilic lymphoid cells of the same type were observed together with normal hepatic glandular epithelium, sparse bile duct epithelium and fairly many Kupffer cells, often loaded with phagocytized material. In a fine-needle biopsy from the spleen cells of the same type were also present.

Lymph node. Lymph node biopsies were done twice and showed essentially the same picture. The normal lymph node structure was completely replaced by a diffuse infiltration of cells of a lymphoreticular type (Fig. 2). The dominating cell was large and lymphoid in character, presumably a large lymphocyte or lymphoblast. Numerous reticulum cells could also be observed. Some of these cells had two or more nuclei, but no typical Reed-Sternberg cells could be identified. A few mature plasma cells and some eosinophilic granulocytes were also seen. The picture was interpreted as a malignant lymphoma, probably Hodgkin's disease. In smears from a fine-needle biopsy done in Dec. 1969, lymphoid or plasmocytoid cells of varying degree of differentiation dominated the picture. Most of them had

a coarse chromatin structure no apparent nucleoli, basophilic cytoplasm and perinuclear zone, but showed great variation in size. In addition more lymphoblast-like cells were present, with reticular chromatin structure and well developed nucleoli (Fig. 2 *b–c*). Most of the remaining cells resembled reticulum cells with one or tw large nuclei, nucleoli and abundant, faintly basophilic cytoplasm, sometimes containing inclusions (Fig. 2 *d*). A few eosinophils were also seen.

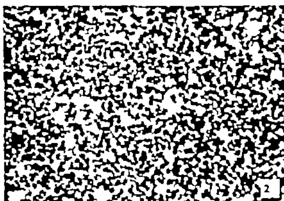
Autopsy findings. The post-mortem examination revealed an extensive generalized lymphadenopathy with large nodes in the axillar inguinal, supraclavicular and submandibular regions, in the mediastinum and along the abdominal aorta. The spleen was enlarged (830 g) and contained infarctions of varying size. The liver was also enlarged (2 070 g). No remnants of the thymus gland could be identified. Microscopically the lymph nodes and the spleen were found to be infiltrated by lymphoreticular tissue of the same type as earlier observed in the biopsies. In some areas the reticulum cells, in some the more lymphoid cell types dominated. Necroses were observed both in the spleen and in the lymph nodes. In some parts there was an admixture of neutrophilic leukocytes, but only few eosinophils ere observed and no typical Reed-Sternberg cells. I the liver dense periportal infiltrations of lymphoreticular cells ere observed.

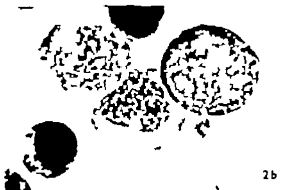
Case 3

Bone marrow Aspirations of sternal marrow from 1963, 1966 and 1970 showed approximately the same picture. The number of lymphocytes ranged from 13 to 21% and they appeared quite normal. An increase of the plasma cells was consistently noted, ranging from 7 to 14%. The plasma cells were mostly of mature kind with only slightly increased polymorphism and in some cells discrete nucleoles (Fig. 3 *a*). In the marrow from 1963 some cells with flaming cytoplasm were observed. Some cells had abundant cytoplasm and more lymphoid character.

Spleen. A fine-needle biopsy from the spleen, performed in Jan. 1964, showed cell picture dominated by lymphocytes and plasma cells (Fig. 3 *b*). The plasma cells were mature and without nucleoli. There was also an increase of mature granulocytes and some reticulum cells.

Liver A fine-needle biopsy made in 1964 revealed normal liver epithelium, but also some small areas with nuclear necrosis, fibroblast invasion and slight lymphocyte infiltration. No plasmocytosis as observed.







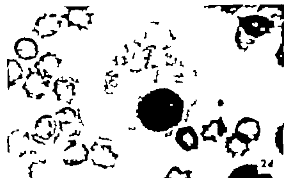


Fig. 2. Case 2. Characteristic morphological features
(*a*) Section of lymph node removed by biopsy showing infiltration of different types of large lymphoid cells and reticulum cells. Van Gieson stain, 175
(*b* and *c*) Large lymphoid or plasmocytoid cells in smear from fine-needle biopsy of lymph node Giemsa stain, 1 512.
(*d*) Large cell with pyknotic nucleus, probably phagocytizing reticulum cell, in smear from fine-needle biopsy of lymph node. Giemsa stain, 952



Fig. 3. Case 3. Characteristic morphological features. (*a*) Plasma cell in bone marrow smear. Giemsa stain, ×1 887.
(*b*) The same type of cell in smear from fine-needle biopsy of the spleen. Giemsa stain, ×1 428.

Immunological Investigations

Immunoelectrophoresis. Immunoelectrophoresis was performed according to the micromethod of Scheidegger (22), using agar gel prepared in veronal buffer pH 8.4. The immunoelectrophoretic examination of serum from all three patients revealed an M-component reacting with antisera against whole γ-chain and the Fc part of the γ-chain, but not with antisera against light chains (Fig. 4). Urine from the three reported patients was concentrated by ultrafiltration before examination by immunoelectrophoresis. In all cases an M-component was observed, showing the same immunoelectrophoretic characteristics as that found in serum.

Gel filtration. Sera from all patients were subjected to gel filtration on Sephadex G-200 in 0.05 M phosphate buffer 0.5 M NaCl, pH 7.0. The eluted fractions were concentrated by ultrafiltration and examined immunoelectrophoretically. In this way the M-components were shown to be present in the albumin-containing fraction (Fig. 5), indicating that the proteins making up the M-components all had a molecular weight well below that of normal IgG. The fraction corresponding to normal IgG was virtually absent in all three cases.

Quantitative immunoglobulin determinations. The levels of IgG, IgA and IgM in serum from the three studied patients were measured using radial immunodiffusion in agar gel according to Mancini et al. (15). Normal serum containing known amounts of immunoglobulins was used as a standard. Normal values of immunoglobulins are in this laboratory using the method described, for IgG 1 323±279 (S.D.) mg/100 ml, for IgA 158±61 (S.D.) and for IgM 88±43 (S.D.). Furthermore, the pathological proteins from patients 2 and 3 were isolated from serum, using gel filtration on Sephadex G-200, followed by electrophoresis in 30×60×1 cm starch blocks in veronal buffer pH 8.6, 0.05 μ. The protein content of these preparations, which were devoid of contaminating serum proteins as judged by immunoelectrophoresis (14), was determined by the Lowry method (13). The purified M-components were then used as references in determining the concentration of the pathological protein in serum and urine of these patients, using the radial diffusion in gel method. The values obtained are shown in Table I.

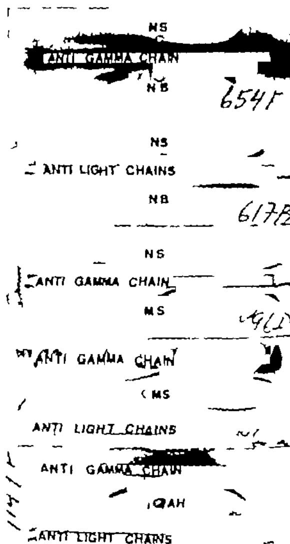


Fig. 4. Normal serum and sera from the patients examined by immunoelectrophoresis with antiserum against γ-chain and antiserum against light chains. Normal serum = NS. Pat. 1 = NB, pat. 2 = MS, pat. 3 = AH.

It is to be noted that the values for IgG are higher when normal serum is used as standard than when purified γ-HCD protein is used in the same way. This is most probably due to the fact that the patients had very little normal IgG (as judged by Sephadex gel filtration and immunoelectrophoresis). In addition the HCD proteins have lower molecular weight than normal IgG hence diffusing faster than normal IgG. This would tend to give erroneously high values when measuring HCD proteins against normal serum standard. The values shown in Table I, where purified HCD protein was used as standard, probably represent the real amount of γ-HCD protein present in the serum and urine of patients 2 and 3.

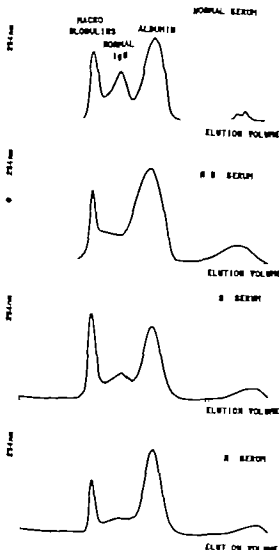


Fig. 5. Gel filtration of normal serum and sera from the patients. The peak corresponding to normal IgG is virtually absent from all three patients sera. Patients 1, 2 and 3 are indicated as in Fig. 4.

Table I. *Immunoglobulin levels (mg/100 ml) in serum and urine of three patients with γ-HCD measured by radial immunodiffusion in gel*

	Case 1	Case 2	Case 3
Values obtained using normal serum as standard			
IgG	11 250	3 975	2 360
IgA	48	21	43
IgM	13	8	30
Values of IgG obtained using purified γ-HCD protein as a standard			
Serum	N.D.	1 300	790
Urine		42	42

Not determined. The concentration of the M-component in serum was approximately 900 mg/100 ml, as determined by paper electrophoresis.

Immunofluorescence. Bone marrow cells from patient 3 were prepared for immunofluorescence as described by Hijmans et al. (9). Antiserum to human γ-chain was labelled with fluorescein-iso-thiocyanate (FITC) and antiserum to human light chains with tetramethyl-rhodamine-iso-thiocyanate (TRITC) as described by the same authors. The bone marrow preparations were treated simultaneously with these labelled antisera, whereafter the preparations were examined in a Leitz microscope, equipped with filters enabling differentiation between FITC and TRITC-labelled cells. It was found that 17% of the bone marrow cells from patient 3 reacted with the labelled antiserum to γ-chains and that only one out of six such cells also reacted with the labelled antiserum to light chains (Fig. 6 *a*, *b*).

DISCUSSION

When the first five cases of γ-HCD were reported in 1964 (6, 20) they seemed to have, besides the rather well defined immunoglobulin abnormality, many clinical and morphological features in common. All cases presented or developed later a generalized lymphadenopathy and most of them had a marked hepatosplenomegaly. The lymph node morphology varied, but in all cases there was some kind of malignant lymphoproliferative process. In some cases reticulum cells of varying maturity dominated the picture, in others a more or less massive infiltration of lymphoid or plasma cells was found. Many of the observed cells appeared to be intermediate forms between immature lymphocytes and plasma cells. The bone marrow contained in all except one case a considerable amount of "atypical" plasma cells, and

the earlier reported cases. The patient, a 70-year old woman, has generally been in good health apart from a tendency to repeated upper respiratory tract and urinary infections. In 1964 a moderate splenomegaly was noted at a routine examination. Both a fine-needle aspiration biopsy from the spleen and a sternal puncture showed an increase of well differentiated plasma cells. Seven years later the patient is still alive and in good health. The sternal marrow contains 8% plasma cells and the splenomegaly is unchanged. No enlarged lymph nodes have been observed. The patient has for some years had a rather advanced osteoporosis in the axial skeleton, but no signs of myelomatous destructions have been observed. The most striking feature is the definite benign character of the clinical and morphological changes observed in this case. No increase has been noted either in the degree of plasmocytosis or in the amount of pathological protein present in the serum or urine. The observed features are except for the splenomegaly rather similar to the findings in cases of "benign essential monoclonal hypergammaglobulinemia" (27) or diffuse (non myelomatous) plasmocytosis with dysproteinemia" (1) Few if any of the earlier described cases had the benign character of this patient. The second case described by Wager et al. (26) had an lated splenomegaly but no morphological de s were reported. One of the first patients Zuc 20) started with a splenomegaly but many years later developed a single lymph node swelling. This case never showed any bone marrow plasmocytosis and ran an apparently benign course.

The immunological findings in our three cases were essentially the same as those earlier described in γ-HCD No definite molecular weight determinations are reported here We do, however consider the results of the gel filtration and immunoelectrophoresis as proof of the existence of a substance in serum and urine of our cases, immunologically related to IgG but with a molecular size below that of normal IgG This substance did not react with antisera against light chains. In cases 1 and 2 the paper electrophoresis showed an elevation of the β-globulins but no definitely abnormal peak. In case 3 a small but distinct M-component was present, located in the slow β-region. In all three cases there was a pronounced suppression of normal immunoglobulins (Table I). In all cases an intermittent proteinuria was observed. In cases 1 and 3 only traces of protein were observed in routine tests, in case 2 the proteinuria in the terminal phase of the disease amounted to 4–5 g/ 4 h. By immunological methods, however the urine could be shown to contain an M-component similar to that found in serum. Further studies of the molecular weights of the purified HCD proteins are in progress and point to the possibility that the pathological IgG fragments in our three cases are larger than the majority of those earlier described.

One of the most interesting questions, both from clinical and morphological standpoint concerning these cases of γ-HCD is whether the occurrence of an M-component, consisting of a fragment of the normal IgG molecule is to be considered only as an accidental finding in cases with a malignant proliferation of immunocompetent cells or whether patients with this type of abnormality represent a uniform disease entity

The clinical syndromes, characterized by the presence of an M-component, show a great variability as do the pathological proteins themselves. In the case of clear-cut myeloma the immunoglobulins synthesized by the pathological cell clone are usually normal immunoglobulin molecules, which in some cases have been shown to be antibodies against defined antigens (21). Some cases of myeloma, however synthesize light immunoglobulin chains only or in excess of heavy chains. Furthermore one case of multiple myeloma has been reported in which the immunoglobulin molecules apparently consisted of "half-molecules i.e. only one light and one heavy immunoglobulin chain (10) In γ HCD on the other hand, the M-component consists of molecules which contain incomplete γ-chains and no light chains. Franklin et al. (6) predicted the existence of immunoglobulin defects, similar to those described in γ-HCD engaging the heavy chains of the other classes of immunoglobulin molecules Cases with such defects in the synthesis or production of monoclonal IgA and IgM have also been described. Seligmann et al. (23) reported the presence of defective IgA heavy chain molecules in one patient with a progressive abdominal lymphoma with a diffuse lymphoplasmocytic infiltration of the small intestine. This immunoglobulin abnormality has later been

shown to exist in further patients with the same clinical picture. This *alpha chain disease* probably represents a true entity defined by characteristic clinical, biological and pathological features. It has, however not yet been shown whether all cases with lymphoma of the digestive tract have this particular defect in IgA heavy chain synthesis or whether this abnormality may be found in other diseases. A disturbance involving the μ-chains of the IgM molecule has also been reported (5) This case was a 58-year-old man with chronic lymphatic leukaemia and amyloidosis, whose serum contained IgM molecules with defective μ-chains in a polymerized state but no light chain determinants. The patient had, however also free kappa chains in serum and urine and it is possible that the defective μ-chains lacked the portion necessary for coupling with the light chains. The clinical picture was not quite typical of chronic lymphatic leukaemia, the patient never developed lymphadenopathy and his bone marrow contained increasing amounts of plasma cells

It is known that in patients with malignant lymphoma (with the remarkable exception f Hodgkin's disease) an increased frequency of M components (usually of IgM type) occurs (11 17 29) Cases have also been published which seem to be transitional between myeloma or macroglobulinaemia and reticulum cell sarcoma (18 34). In some of these cases there has been a predominance of plasma cells together with the proliferation of reticulum cells. In the hitherto published reports it has, however not been possible to separate those cases which ha e been producing monoclonal immunoglobulins on morphological grounds. This can be explained by the fact that light microscope examination of these cells as a rule is not sufficient to determine whether they possess the capacity of immunoglobulin synthesis.

The features that clinically characterize most of the hitherto observed 14 cases of γ-HCD are generalized malignant lymphoma, hepatosplenomegaly and symptoms presumably caused by the defective production of normal immunoglobulin such as recurrent infections and septic episodes. In some cases a peculiar swelling and erythema of the uvula and palate has been observed and compared to the findings in cases with infectious mononucleosis (19) Some of the patients have, however run a different and more benign course and at least our patient 3 is still alive and in good health. On purely clinical grounds the more malignant cases ha e as a rule been regarded as generalized lymphomas; in the benign cases the symptomless splenomegaly has been the outstanding feature. The morphological investigations have, however in most of the cases, revealed the presence of an infiltration in the lymphoreticular organs of plasmocytoid cells of varying maturity probably responsible for the production of the monoclonal immunoglobulin.

Considering the facts discussed above, it seems to us probable that, in the broad spectrum of states with a more or less malignant proliferation engaging the reticulo-lympho-plasmocytoid cell system, now and then a clone of cells may arise, capable of immunoglobulin synthesis and secretion. These cells have as a rule had the appearance of normal Ig-producing cells resembling plasma cells of varying maturity In some cases, however the light microscopic features of the dominating cell type ha e been those of a small lymphocyte or an "atypical" or plasmocytoid" lymphocyte with more abundant and basophilic cytoplasm. In other cases most of the cells ha e had the appearance of immature reticulum cells. Electron microscopic studies of the cells concerned have only been undertaken in a few cases (32, 33) but have then shown a rather well developed endoplasmic reticulum, well in correspondence with the findings in other cells engaged in immunoglobulin synthesis Why these cells in some cases produce complete immunoglobulin molecules, in some only fragments of the normal IgG IgA or IgM molecule or pathological fragments, or why in yet some a defect arises in the assembly of the different parts of the molecule, are questions still unanswered The f ct that the observed immunoglobulin fragments in our patients seem to be larger than those earlier reported is well in accordance with the theory that the type of immunoglobulin molecule produced may vary from one case to another depending on the properties of the clone f cells proliferating

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TREATMENT OF HEREDITARY ANGIONEUROTIC OEDEMA WITH TRANEXAMIC ACID

A Random Double-blind Cross-over Study

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Abstract. Five patients with hereditary angioneurotic oedema (HANE) have been treated, in a random double-blind cross-over study with tranexamic acid (trans-AMCHA). Three patients have reacted positively to the treatment. The positive effect in these cases has been so obvious that a specific effect of trans-AMCHA seems probable even if it cannot be definitely ruled out that spontaneous variations have been the cause of this effect. Therapeutic trials with fibrinolytic inhibitors should be made on patients with HANE.

Hereditary angioneurotic oedema (HANE) is a disease that has become of increasing interest during the latest decade. In 1963 Donaldson and Evans (9) demonstrated an abnormality in the complement system in patients with this disease. This abnormality is related to a deficiency of the normal plasma inhibitor of C1 esterase, one of the nine known components in this system. It has now become possible to prove the deficiency of the inhibitor by enzymatic and immunochemical methods (19). The disease is characterized by attacks of local oedema appearing in the skin and in the mucosa of the upper respiratory and the gastrointestinal tracts. When localized to the upper respiratory tract, breathing difficulties not infrequently occur which may lead to asphyxiation. When the gastrointestinal tract is involved, this may lead to severe abdominal pains and vomiting. The attacks not infrequently occur with pronounced periodicity. The degree of severity of the symptoms and the frequency of the attacks vary from case to case. This has been shown in an investigation of three families from West Sweden consisting of 21 now living members with this disease (5).

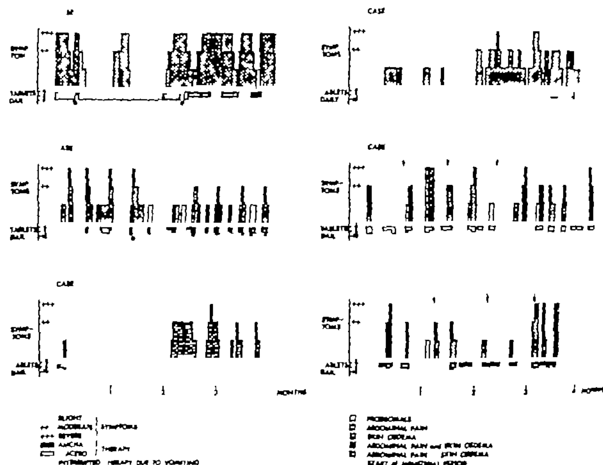
Many drugs have been tried as treatment for HANE. Treatment with calcium, antihistamine, adrenaline, corticosteroids, chlorpromazine and oestrogens have not been able to prove any definite effects. In 1959 Spaulding (27) introduced treatment with methyl testosterone for this disease. He treated five patients during a period of $^1/_2$–6 years with obvious effect. Since then a few further cases have been treated with apparently good symptomatic effect (9, 17, 24). In 1966 Nilsson et al. (23) reported a positive effect with oral doses of an antifibrinolytical substance, epsilonaminocaproic acid (EACA), given intermittently to a patient with HANE. This led to EACA and the more potent tranexamic acid (trans-AMCHA) being tried on a further number of cases with this disease with, however, varying results (5, 6, 15, 17, 20).

An investigation with random double-blind cross-over technique of an antifibrinolytical substance for HANE was therefore thought to be justified. Trans-AMCHA (Cyklokapron®, supplied by Kabi, Stockholm) was chosen as the substance.

MATERIAL AND METHODS

Six patients, five women and one man, were chosen for this test from three families with HANE. These families have earlier been referred to in detail (5). One woman did not take the tablets according to the instructions and did not keep any diary, therefore the effect of trans-AMCHA on her symptoms could not be estimated. In four of the five remaining cases (cases 1–4) complement studies have earlier been carried out which verified the diagnosis (5). In this earlier publication the three families have been called A, B and C. Cases 1 and 2 in the present study belong to family A, cases 3 and 4 to family B and case 5 to family C.

The investigation was carried out as a random double-



1 Symptoms and therapy in cases 1 5

and cross-over study Cases 1–4 were treated over periods of tw months with either placebo or trans-AMCHA. The corresponding period in case 5 was four months. Two patients started ith trans-AMCHA and three with placebo. Both types of tablets ere similar in appearance and taste and were supplied in similar bottles. The active tablets contained 0.5 g trans-AMCHA. Every day the patients registered the number of tablets taken, HANE symptoms and eventual side-effects. The numbers of tablets taken, as registered by the patients themselves, are show in Fig 1 The dose aried from patient to patient because of reasons discussed in the case reports. Three patients (nos. 1 3 and 4) received con tinuous treatment with a dose of 2–3 tablets three times daily T patients (nos. 2 and 5) received intermittent treatment of 2 3 tablets 3 times daily for couple of days in conjunction with attacks The treatment of these patients started at the first sign of an attack of HANE. The patients were informed that t o equivalent preparations ere to be tested.

CASE REPORTS AND RESULTS

Case 1

A 49-year-old oman ho had HANE symptoms since she was one year old. She had recurrent oedema of the skin, sometimes together with oedema of the larynx and disabling abdominal pains and vomiting. The symptoms occurred with approximately the same frequency through the years. No definite periodicity in the symptoms was noted. Mild attacks lasted 3–7 days and the intervals between the attacks during her best periods were –4 weeks. In between, the symptoms were continuous for several months. Longer intervals than one month between tw attacks had not occurred during the latest 10-year period. Treatment with calcium, adrenaline, antihistamine and steroids had had no effect. Some months before the investigation she had, more or less, constant severe symptoms. The patient received continuous treatment with dose of 2–3 tablets 3 times daily for four months. Placebo was given during the first -month period and trans-AMCHA during the second. Symptoms and treatment are shown in Fig. 1.

During the first two placebo weeks the patient experienced no improvement. On one occasion the treatment had to be withdrawn, because of vomiting. In the following six weeks the patient had only one semi-severe attack. Two days after she began the treatment ith trans-AMCHA, abdominal pains and oedema of the skin returned. These symptoms remained, more or less severe, during the whole period in which trans-AMCHA as given. With a daily dose of nine tablets (i 4.5 g

trans-AMCHA daily) the patient reported side-effects in the form of nausea and slight headache. These side effects diminished when the dose was reduced to six tablets (3.0 g) daily. On four occasions during the treatment period with trans-AMCHA the therapy had to be withdrawn because of vomiting and severe abdominal pains. When the treatment was stopped the symptoms continued with undiminished intensity for about month. The code had, at this point, not yet been broken, but at the patient' urgent request tablets containing trans-AMCHA (Cyklokapron® Kabi) were again given in dose of 3.0–4.5 g daily. The patient has, since then, continuously taken this preparation during the past year as she considers it help. She states that, although the attacks appear as often as before, the symptoms are considerably less pronounced. This is true for both the abdominal symptoms and the oedema of the skin.

The patient noted no respiratory difficulties during the trans-AMCHA therapy. Before treatment with trans-AMCHA the patient had laryngeal oedema every to every other month. Initially Cyklokapron® in dose of 4.5 g daily caused heartburn, flatulence and diarrhoea. However, during continued treatment for some time only slight diarrhoea remained. No effect on white cell and platelet counts or liver and kidney function tests have been observed.

Case 2

A 48-year-old man who had HANE symptoms since six years of age. He had, with pronounced periodicity attacks of abdominal pains with vomiting, occasionally together ith oedema f the skin. Sometimes oedema of the larynx also occurred. The attacks, in recent years, appeared at intervals of two weeks and f such severity that the patient had to remain in bed for 1 2 days. During attacks he often noted heartburn and was hospitalized several times for peptic ulcer disease with haemorrhage. This patient had extremely high gastric secretion of hydrochloric acid, as has been reported earlier (13). During the three years prior to the present double-blind study the patient had been treated partly continuously and partly intermittently with EACA (Epsikapron® Kabi) with good effect, as previously reported (5). During the past two years EACA was given in the form of suspension. The dosage was 3–6 g EACA 2–6 times daily for 1–4 days beginning at the *first sign* of attack—as rule every 14th day. In this way the attacks ere as rule completely prevented. On few occasions, when the treatment as not immediately started at the first signs, typical attacks occurred.

The double-blind investigation was carried out as intermittent treatment with this patient. He was recommended to take three tablets at the first sign of an attack and later three tablets 3 times daily for 1 3 days. Symptoms and treatment during the periods are show in Fig. 1. The patient received placebo as first drug. During the placebo period four definite severe attacks of HANE ere noted, following the typical course ith abdominal pains and vomiting but without oedema of the skin. The therapy had to be stopped every time because vomiting. On all these occasions the patient was lying in bed 1 days. During the latter part of the placebo period heartburn occurred ith an increased frequency between the proper attacks. During the period with trans-AMCHA the patient noted altogether nine attacks, three of hich ere only in the form f prodromes whilst six developed into slight to semi-severe attacks.

The patient reported positive effect of the treatment on each attack and could work to the full extent during this treatment period. Compared to his previous treatment with EACA he considered, however that the present treatment was less ffective. The patient considered that the effect was the same as when EACA was given slightly later in the initial phase of an attack. After the double-blind study the patient returned to the earlier regime with intermittent EACA therapy with just as good results as previously. No effect on hite cell and platelet counts or liver and kidney function tests have been observed.

Case 3

A 73-year-old woman ho had HANE symptoms since six years of age. Her attacks of abdominal pains and vomiting, sometimes together with oedema of the skin and larynx, showed pronounced periodicity with intervals of tw eeks between the attacks. In recent years the abdominal pains occurred most often. During the latest fiv years the symptom-free periods between attacks had not been longer than 2–4 eeks. Oedema of the skin and larynx occurred 1 / months before the actual double-blind study. This patient received continuous treatment with dose of tw tablets three times daily for four months. In this case lower daily dosage of trans-AMCHA than in case 1 was prescribed because, earlier extensive muscle necrosis had been seen during EACA therapy in case from this family (17). Symptoms and treatment are show in Fig. 1. The patient began the treatment with trans-AMCHA.

After three days with trans-AMCHA treatment the patient noticed slight oedema of the skin and took two extra tablets. The swelling disappeared during the day. During the remaining part of the trans-AMCHA period the patient was completely free from the typical HANE symptoms. During the first two weeks of the trans-AMCHA period she noticed slight increase in flatulence and slight diarrhoea. One week after beginning the placebo period she had recurrence of typical attacks of abdominal pains, nausea and oedema of the skin with 2-week intervals.

After the double-blind study the patient has been treated with Cyklokapron® for the past year in dose of tw tablets 2 3 times daily. During this time she had five slight attacks. No effects on hite cell and platelet counts or liver and kidney function tests have been observed.

Case 4

A 56-year-old oman, ho had HANE symptoms since six years of age. She is sister to case 3. She had attacks of abdominal pain ith vomiting and oedema of the skin, sometimes together with oedema of the larynx. The attacks occurred with 1 2-week intervals. In recent years the abdominal symptoms ere dominant. During the four months prior to the actual double-blind study she received Cyklokapron® continuously in an oral dose of

1.5–2 g daily With this dosage she reported that the symptoms had almost disappeared. The dosage during the study was tw tablets three times daily for four months. Symptoms and treatment during the periods are shown in Fig. 1 The patient began the treatment with trans-AMCHA.

During the trans-AMCHA period the patient noticed ery slight attack, with moderate abdominal symptoms lasting couple of days, and on two occasions a vague slight discomfort in the abdomen. At the beginning of the treatment she noted the ppearance of slight diarrhoea. Two days after the beginning of the placebo period her abdominal symptoms returned and remained during the whole placebo period with varying intensity At the end of this period, because of severe abdominal pains, she took two tablets of Cyklokapron® which coincided with an improvement in her condition. After the end of the period she reverted to the earlier treatment with Cyklokapron® hereupon the symptoms again diminished.

After the double-blind study she has been treated with Cyklokapron® for the past year in dose of two tablets 2–3 times daily During this year she had had no symptoms. No side-effects apart from slight diarrhoea have been noted and no effect on white cell and platelet counts or liver and kidney function tests have been observed.

Case 5

A 48-year-old oman ho had HANE since four years of age. The main symptoms were oedema of the skin and larynx, although abdominal symptoms had also occurred relatively often. During adolescence she f equently suffered from life-threatening oedema of the larynx. Her cks of oedema of the larynx had successively di- thed in frequency through the years. Attacks of NE often occurred during menstrual periods. In the year prior to this investigation the patient had attacks of HANE during every menstrual period. She lso noted typical prodromal symptoms such as tiredness and headache, which meant that she could easily predict coming attack. Therefore the patient received intermittent treatment during two 4-month periods with initially 3 tablets at the first sign of an ttack and thereafter 3 tablets 2 3 times daily for 2 5 days. She was also recommended to take the same dose of tablets just prior to and during the first two days of each menstrual period even in the absence of prodromal HANE symptoms. Symptoms and treatment during the periods re shown in Fig. 1 The patient began with placebo.

Initially during the first month of placebo treatment, the patient reported apparent side-effects ith constipation and a feeling of distension of the abdomen with dosage of three tablets three times daily Therefore, the dose was reduced to two tablets 2 3 times daily which could be tolerated by the patient No further side-effects have appeared either during the placebo or the trans-AMCHA periods.

During the placebo period the patient noted 11 attacks and on one occasion only prodromal symptoms During four of the attacks there was only oedema of the skin, during tw only abdominal symptoms, and during five combination of both. The patient stated that the treatment had a certain effect on the degree of severity of the ttacks during the first and third months, whilst no effect was observed during the second and fourth months. During the trans-AMCHA period the patient noted nine attacks and on one occasion only prodromal symptoms. During six of these attacks there was only oedema of the skin, during one only abdominal symptoms, and during two a combination of both. The patient considered that the treatment was partly effective during the seventh month but not during the fifth, sixth or eighth. At the end of the eighth month the abdominal pains and above all the skin oedema were reported to be more pronounced than usual.

DISCUSSION

The character of periodic disease, the location of oedema to the laryngeal region and the often lifelong distress in HANE have initiated many therapeutic trials with numerous drugs during the past years. Not until methyl testosterone was shown to have a prophylactic effect (27) were these trials successful. Unfavourable side-effects such as jaundice acne, gynecomastia and facial hirsutism (27) have limited the value of methyl testosterone. The introduction of EACA and later trans-AMCHA two antifibrinolytical agents, in the therapy of HANE brought therapeutic alternatives. Only a few studies on isolated cases have been reported (5 6 15 17 20) The results are conflicting. The urgent request for an effective drug without serious side-effects for long-term treatment of patients with HANE prompted us to further elucidate the effect of trans-AMCHA on the symptoms of HANE.

It is well known that attacks of HANE vary from patient to patient with regard to combination of symptoms, degree of severity and frequency Even in the same person there exist remarkable differences from one attack to an other (5). It is not possible to make a traditional controlled study in a disease of this type. The experimental design and dose schedules were therefore modified individually

Three of five patients considered trans-AMCHA to have a positive effect. Two of them had continuous and one intermittent therapy In family A one patient (case 2) considered the effect positi e. The other patient in this family (case 1) reported, on the contrary a deterioration of her disease during the trans-AMCHA period. Two genetic variants of HANE have been described (26). The majority of families were

shown to have a real deficiency of inhibitor of C1 esterase, but in a small number of families there was a normal amount of non-functioning inhibitor protein present. Complement analysis from affected members of the three studied families showed the same abnormality with low values of inhibitor protein (5). No differences in complement factors could thus be found to explain the therapeutic differences.

It is still not clear how the unique deficiency of functioning inhibitor of C1 esterase affects the initiation, course and frequency of attacks of HANE.

The complement system consists of nine hitherto known serum components activating each other in a strict sequence (21, 22). This activation results in split products (7, 14). Such activation has frequently been discussed as the main reason for the symptoms (3, 10, 12). An abnormality in the histamine metabolism could also be involved (2, 13, 20). Kinins have been discussed as mediating factors and Donaldson et al. (10) have recently partially isolated a protein from HANE plasma which produced a pronounced increase in the vascular permeability. The fibrinolytic enzyme plasmin is blocked by the inhibitor of C1 esterase (25). It has been suggested that plasmin may trigger the activation of C1 to C1 esterase (9).

EACA and trans-AMCHA are synthetic fibrinolytic inhibitors that function by inhibiting the activation of plasminogen to plasmin (1). The inhibitory effect of trans-AMCHA on plasminogen activation by tissue activators and urokinase has been reported to be about ten times greater than that of EACA (1). In higher concentrations trans-AMCHA also inhibits the activation of trypsinogen by enterokinase, trypsin and pepsin (11). EACA is considered to have a moderate inhibiting effect on the activation of C1 esterase (8, 28). EACA is also considered to have a moderate inhibiting effect on the kinin-releasing activity of trypsin and kallikrein, as distinguished from trans-AMCHA which show little effect on any of these enzymes (4).

EACA and trans-AMCHA may thus influence several enzyme reactions in the body. It is not proven that these substances have a direct effect on all symptoms in HANE, but there are strong indications that such an effect could modify the symptoms in an individual case. The possibility exists that symptoms from the gastrointestinal tract are released after reactions in a somewhat different enzyme chain than is the case when oedema of the skin appears. An observation indicating this is that the best effect of trans-AMCHA was attained in patients in whom gastrointestinal symptoms dominated.

The difference in effect between EACA and trans-AMCHA in case 2 may be due to spontaneous variations in the course of the illness and/or changed experiences of symptoms during this study. Another explanation of the difference could be that the speed of absorption from the gastrointestinal tract is considerably higher for EACA than for trans-AMCHA (16). In this study trans-AMCHA was given in tablet form, which might further have reduced the speed of absorption. It has earlier been observed that antifibrinolytical treatment in the intermittent therapy of HANE must be given as quickly as possible at the first prodromal symptoms (5, 23). The possibility that differences in action of the two inhibitors may explain the observed difference in the effect cannot be excluded.

Case 1 showed a pronounced deterioration in her illness during the trans-AMCHA period compared with the placebo period. This deterioration may be the result of a spontaneous variation. It cannot be ruled out, however, that trans-AMCHA initiated new symptoms, as the attacks recurred only two days after the start of the trans-AMCHA period. Case 5 as well reported an accentuation of both oedema of the skin and abdominal pains during the latter part of the trans-AMCHA period. She showed, however, a pronounced spontaneous variation during the four months of the placebo period, therefore a deteriorating effect of trans-AMCHA seems to be less probable. Juhlin and Michaelsson (15) obtained deterioration in two women with HANE during two weeks' treatment with 18 g EACA daily and 2 g trans-AMCHA daily.

No serious side-effects have been noted during the treatment periods. One patient suffered from nausea and slight headache with a dose of 4.5 g trans-AMCHA daily. Three patients reported slight transient diarrhoea. There are only a few reports on side-effects during long-term treatment with fibrinolytic inhibitor. One patient, son of case 4 in this study with severe symptoms of HANE, developed extensive muscle

he was successfully treated with EACA in an oral dose of 30 g daily (17) One patient with HANE got a prostatovesiculitis during EACA therapy that healed and disappeared when changing the therapy to trans-AMCHA (20)

This investigation has not proved a definite effect of trans-AMCHA on HANE symptoms in all cases. There is strong evidence, however that fibrinolytic inhibitors, in certain cases, have a positive effect. The present results indicate that, in patients with HANE, therapeutic trials should be made with fibrinolytic inhibitors. Both EACA and trans-AMCHA should be tried. In patients with symptoms of pronounced periodicity an intermittent therapy could be sufficient to control the attacks.

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STROKE IN HYPERPARATHYROIDISM

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Abstract. Twelve patients with signs and symptoms indicative of major stroke and five patients who had suffered from syncope and other signs of minor stroke were found to have coexistent hyperparathyroidism (HPT) been admitted to hospital as emergency cases. The mean age of the former group, consisting of three males and nine females, was 69.7 years (range 53–84). The mean age of the second group two males and three females, was 66 years (range 56–84). Fourteen patients were operated on. In ten of them parathyroid adenoma was found, in three primary parathyroid hyperplasia, in one parathyroid cancer. Three patients died before parathyroid exploration could be undertaken. Parathyroid adenomas were revealed at autopsy Of the operated patients one died of a new stroke 1 month after operation and another of metastasis of parathyroid cancer The neurological and mental condition of all the 12 surviving patients improved. The possible relation between the development of signs of major or minor stroke and HPT is discussed. The importance of normalization of the parathyroid function in these patients, in order to facilitate the rehabilitation of the patients with major stroke, is stressed. In patients with minor stroke and HPT early operation might prevent development of major stroke.

The wide variation in the clinical picture of primary hyperparathyroidism (HPT) is now well recognized. Not only the classical manifestations of typical bone (4 21 26) kidney (1 7 8) and gastrointestinal diseases (22, 24) are known to be associated with HPT but also a series of other severe manifestations of systemic disease e.g. hypertension (11 16) psychoneurological disorders (12, 14 18) joint lesions (6) and myopathy (5, 15, 26) have been shown to be related to HPT in a large number of cases. On the other hand reports of more severe manifestations of cardiocerebrovascular diseases, e.g. cardiac infarction and stroke occurring in patients with an established diagnosis of HPT are extremely rare.

The scarceness of such reports is somewhat surprising since hypercalcemia, induced by the administration of excess parathyroid hormone or vitamin D to experimental animals, is known to affect profoundly the metabolism of connective tissues, including those of the vascular walls (19 20)

From 1 Jan., 1966, to 31 March, 1971 we have studied and treated 170 patients suffering from primary HPT A significant number of these patients, mainly elderly women, have suffered from a major stroke immediately before hospitalization, or during a hospitalization period at which the diagnosis of HPT was established In fact, in some other cases one or several attacks of syncope suggestive of a minor stroke constituted the presenting symptom. The scope of the present paper is to report briefly on the clinical course *of these patients, to discuss the possible relation* between major and minor stroke and HPT when occurring together and to stress the importance of treatment of coexisting HPT in order to facilitate the rehabilitation of patients with sequelae of major strokes.

MATERIAL AND METHODS

The present study is based on a review of the following groups of patients.

Group I This group included 170 patients with HPT referred from various hospitals to the Department of Surgery of Serafimerlasarettet in Stockholm, for exploration of the parathyroids, between 1 Jan., 1966, and end of March, 1971

Group II This group included 12 patients with HPT in whom the diagnosis was made after screening for hypercalcemia 268 consecutive patients admitted to Medical Department III of St. Erik Hospital in Stockholm between 1 April, 1969 and 31 Dec., 1969

Table I. *Age and sex distribution of 12 patients with HPT and signs of major stroke and parathyroid histology*

	Total	Operated	Autopsied: Not operated	Autopsied: Operated	Parathyroid histology: Adenoma	Parathyroid histology: Primary hyperplasia	Parathyroid histology: Cancer
Females, mean age 71.6 (51–84)	9	6	5	2	6	2	1
Males, mean age 63.6 (62–69)	3	3	0	0	2	1	
Total, mean age 69.7 (51–84)	12	9	5	2	8	3	1

Group III. This group contained two patients with HPT in whom the diagnosis of HPT was established after screening for hypercalcemia 86 consecutive patients, with the presenting symptom of hemiparesis admitted to Medical Departments I, II and III of St. Erik Hospital between 1 April, 1969 and 31 Aug., 1970.

Owing to the close cooperation between the departments involved, some of the individual patients with HPT belonged to t or all three of the above mentioned groups, as indicated in the case report below.

The diagnosis of HPT in the present study is based on at least one of the following groups of diagnostic criteria.

A. Demonstration of significant, consistent hypercalcemia, resistant to treatment with prednisolone (30 mg/ for 10 days) in patients in whom a careful clinical ...ation failed to give any other explanation of the ...

B. Demonstration of parathyroid adenoma, parathyroid *hyperplasia or parathyroid cancer macroscopically and* microscopically in connection with surgical exploration of the neck region.

C. Establishing of normocalcemia after removal of adenomatous, hyperplastic or carcinomatous parathyroid tissues.

D. Demonstration at autopsy both macroscopically and microscopically of parathyroid adenoma, parathyroid hyperplasia or parathyroid cancer.

The laboratory procedures for the determination of serum concentration of calcium, phosphorus, creatinine, albumin, total protein, and alkaline phosphatase, which were performed and carefully evaluated in all patients with one exception (case 5), consisted of conventional methods applied to the Autoanalyzer (25) or in all screening studies at St. Erik Hospital to the Autochemist (17).

The technique of surgery. General anesthesia with endotracheal intubation was used in every operation. The exploration was conducted from the level of bifurcation of the common carotid artery downward into the upper part of the mediastinum. In two patients with recurrence of hypercalcemia a sternal splitting and an extensive exploration of the mediastinum were carried out. In each case attempts were made to identify at least 4 parathyroids. When parathyroid adenoma was found, the whole gland together with the adenoma was removed. Biopsy specimens were taken from all identified parathyroids and examined in frozen sections during operation. Parathyroid tissue corresponding to 1-2 normal glands was not removed in the three patients with primary parathyroid hyperplasia. All the glands were removed and only small pieces corresponding to 1-2 normal parathyroid glands were autotransplanted to the sterno-cleido-mastoid muscle. In this manner a new exploration of the parathyroids would be facilitated in the event of recurrence of the disease. Vitamin D_2 was given postoperatively to these three patients. Only one patient needed this substitution for more than 3 months postoperatively to maintain normal serum calcium.

RESULTS

Of the 170 patients in group I ten had a history of and presented signs indicative of a major stroke preceding the establishment of the HPT diagnosis. Clinical details of these patients are given in case reports 1–10 below.

Of 2 268 consecutive internal-medical emergency cases screened for hypercalcaemia and with a careful clinical evaluation of all cases with significantly elevated serum calcium values, the diagnosis of HPT was established in 12 (group II). This corresponds to an incidence of HPT of about 0.5% in an unselected group of internal-medical emergency cases. In three of the 12 patients with HPT in this group the presenting symptoms and signs indicated a major stroke (case reports 9, 10 and 11).

Of 86 consecutive patients presenting symptoms and signs of major stroke, and subjected to screening for hypercalcemia and a detailed clinical evaluation, the diagnosis of HPT was established

in two (nos. 11 and 12). HPT was not a complication in any of the 49 patients with hemorrhagia cerebri. However, primary HPT was present in one of 18 patients considered to have embolia cerebri, and in one of 17 patients with thrombosis cerebri.

A total of 12 patients presented a combination of major stroke and HPT. Some clinical data on these patients are summarized in Tables I–V. Table I gives the sex and age distribution of these patients, and also shows that in all cases the HPT diagnosis was morphologically verified either by operation or by autopsy. The Table also shows the distribution of the various morphological changes in the parathyroids, adenoma, hyperplasia, and cancer, correlated to the hyperactivity of the parathyroids in the present material.

Table II gives the first preoperative serum calcium value recorded on admission to the hospital where the HPT diagnosis was established. The postoperative level of serum calcium is also shown.

As recorded in Table III, nine of the patients were affected by consistent or (in some cases) transient hemiparesis on the right side. In three patients the hemiparesis was localized on the left side.

In most cases the first attack of hemiparesis occurred immediately before admission. In some of the cases, however, the hemiparesis preceded the establishment of the HPT diagnosis by months or even years. The psychic state of the patient on admission and at operation after conventional treatment of the hypercalcaemia and other relevant electrolyte disturbances is indicated in the Table.

Table II. *Pre- and post-operative calcium levels and parathyroid histology in 12 patients with HPT and major stroke*

Pat. no.	Age (y.)	Sex	Pre-operative Ca level	Post operative Ca level	Parathyroid histology
1	51	♀	6.7	Normocalcaemia	Adenoma
2	62	♀	6.2	Hypercalcaemia	Cancer
3	72	♀	6.2	Normocalcaemia	Adenoma
4	73	♀	8.0		Adenoma
5	74	♀	5.8		Adenoma
6	75	♀	6.2	Normocalcaemia	Adenoma
7	75	♀	8.4	Normocalcaemia	Adenoma
8	78	♀			Adenoma
9	84	♀	6.2	Normocalcaemia	Adenoma
10	62	♂	6.7	Normocalcaemia	Adenoma
11	62	♂	6.0	Vitamin D_2 substitution	Hyperplasia
12	67	♂	6.6	Normocalcaemia	Adenoma

Table III. *Localization and duration of hemiparesis and preoperative psychic condition in 12 patients with HPT and major stroke*

	Hemiparesis		Psychic status	
Pat. no.	Localization	First attack, time before admission	On admission	Preoperatively
1	Right	A few hours	Comatose	Confused
2	Left	A few hours	Comatose	Alert
3	Right	14 years	Alert	Alert
4	Right	A few hours	Alert, slow cerebration	
5	Right	A few hours	Alert	
6	Right	A few hours	Alert, depressed	Alert, depressed
7	Right	1 month	Stuporous	Confused
8	Left	A few hours	Stuporous	
9	Right	4 years	Alert	Alert
10	Left	A few hours	Confused	Alert
11	Right	12 years	Alert, depressed	Alert, depressed
12	Right	1½ months	Slight disorientation	Alert

Table IV shows the tentative neurological diagnosis and cardiovascular diagnosis based on clinical findings (physical and neurological examination, ECG, encephalography, etc.). In some cases the neurological diagnosis was supported by anatomical findings made by means of carotis angiography, encephalography or at autopsy.

Table V summarizes the estimated clinical effects of the operation for HPT on the neurological condition of the patients after various postoperative observation periods.

In five out of 170 patients with HPT one or more episodes of syncope had occurred immediately before admission to the hospital where the diagnosis of HPT was established (case reports 13–17). Three of these patients were females, mean age 71.3 years, and two males, both 72 years of age. Pre- and postoperative calcium levels are given in Table VI, which also shows that parathyroid adenoma caused HPT in four patients and primary hyperplasia in one.

Tentative neurological and cardiovascular diagnoses based on clinical evidence are given in Table VII, as well as an estimation of the clinical effect of the parathyroid operation, and the postoperative observation periods.

Table IV *Neurological and cardiovascular diagnoses and morphological documentation in 12 patients with HPT and major stroke*

Pat. no	Neurological diagnosis	Cardiovascular diagnosis	Remarks
1	Embolia cerebri	Mitral valvular disease + atrial fibrillation	
2	Ischemia cerebri	Cardioarteriosclerosis levis	Carotis ngiography autopsy
3	Hemorrhagia cerebri	Infarctus cordis + hypertonia	
4	Encephalomalacia (embolia) cerebri	Cardioarteriosclerosis levis	Autopsy
5	Embolia cerebri	Cardioarteriosclerosis gravis + diabetes mellitus	Autopsy
6	Ischemia cerebri transitoria + *encephalomalacia cerebri?*	Cardioarteriosclerosis	Carotis angiography
7	Ischemia cerebri transitoria	Cardioarteriosclerosis	
8	Thrombosis cerebri	Cardioarteriosclerosis	Autopsy
9	Ischemia cerebri transitoria	Cardioarteriosclerosis + bradycardia transitoria	
10	Ischemia cerebri	Cardioarteriosclerosis levis	Carotis angiography
11	Ischemia cerebri transitoria	Cardioarteriosclerosis levis	Carotis angiography
12	Thrombosis cerebri	Cardioarteriosclerosis + hypertonia levis + diabetes mellitus	Carotis angiography

CASE REPORTS

Patients with Major Stroke and HPT

Case 1

Female, born 1917. Six years bef e admission in 1968 the patient suffered from a major stroke and flaccid, and subsequently spastic, paresis supervened on the right . At that time the diagnosis was *mitral vitium* and fibrillation. On the day of the actual admission ... ent suddenly lost consciousness and was brought the emergency ward. On arrival she was still unconscious, but regained consciousness after / hour. *Physical examination* showed spastic paresis of the right side and conjugate deviation to the right. She also had signs indicative of combined mitral vitium, and auricular fibrillation her BP was 160/115 mmHg.

Laboratory tests indicated a highly significant hypercalcemia. Operation, with exploration of the *parathyroids*, was performed. Phosphate treatment was given. A parathyroid adenoma was found and removed. The postoperative course was uneventful, and an improvement of the mental and neurological condition was observed. One month after operation, however she died suddenly. Autopsy revealed a hemorrhage in the right basal ganglia.

Diagnostic criteria ABC. Group I.

Case

Female, born 1907. The patient was previously in good health. During the autumn of 1969 however she and her

Table V *Estimated clinical effect of operation and length of postoperative observation period in 12 patients with HPT and major strok*

Pat. no.	Clinical effect of HPT operation: Neurological condition	Psychical condition	Postoperativ observation period	Remarks
1	Improved	Improved	1 mo.	Sudden death f om new stroke
	Not improved	Not improved	3 mo.	Consistent hypercalcemia, death f om parathyroid cancer
3	Improved	No difference	9 mo	
4	Not operated	Not operated		Sudden death
5	Not operated	Not operated		Sudden death
6	Improved	Improved	2 y	
7	Improved	Improved	½ y	
8	Not operated	Not operated		Sudden death
9	Improved	Improved	1 mo.	
10	Improved	Improved	2½ y	
11	Improved	Improved	1 y 9 mo.	
12	Improved	Improved	2 mo.	Postoperative complications

Table VI. *Pre- and postoperative calcium levels and parathyroid histology in five patients with HPT and syncope*

Pat. no.	Age (y.)	Sex	Preoperative Ca level	Postoperative Ca level	Parathyroid histology
13	56	♀	7.4	Normocalcemia	Hyperplasia
14	73	♀	6.6	Normocalcemia	Hyperplasia
15	83	♀	7.1	Normocalcemia	Adenoma
16	72	♂	5.8	Normocalcemia	Adenoma
17	72	♂	6.0	Normocalcemia	Adenoma

relatives noticed gradual loss of memory. Physical examination showed normal findings. The patient had slight sinus tachycardia of 90 beats/min and BP of 135/65. However, consistent and significant hypercalcemia was found to be present, and the diagnosis of HPT was established. Operation, with exploration of the parathyroids, was performed in two steps on 4 Feb. At the first step, undertaken after preoperative phosphate treatment, four normal glands were detected. Hemithyroidectomy and subtotal parathyroidectomy were performed. Inclusions of parathyroid tissue, infiltrating section of the left thyroid lobe, were observed and considered to be indicative of parathyroid cancer. The diagnosis was subsequently established on examination of the paraffin section. Postoperatively the patient became normocalcemic for few weeks and improved mentally. Reoperation with sternal splitting and total parathyroidectomy was performed on 24 March. The hypercalcemia continued. Vigorous phosphate treatment was started, but the patient became unconscious and died. Autopsy revealed tumor of the left ovary with histological structure very similar to parathyroid tissue.

Diagnostic criteria ABD. Group I.

Case 3

Female, born 1898. The patient, German refugee from 1939 was found to be suffering from hypothyroidism in 1955. Since then she has been treated with desiccated thyroid. In 1956 hemiplegia of the right side developed, which slowly improved during the following 6 months. In Oct. 1969 cardiac infarction occurred and she was treated in hospital for two months. During this hospitalization significant hypercalcemia was observed, and the diagnosis of HPT was established. Operation, with exploration of the parathyroids, was performed in July 1970 and an adenoma was removed. The postoperative course was uneventful.

Diagnostic criteria ABC. Group I.

Case 4

Female, born 1895. At the age of 45 the patient was subjected to uterus amputation for benign cyst. Otherwise she had been fairly healthy. However during the last few months before admission her son had noticed that she was more nervous, aggressive and uneasy than previously. Recently she had also complained of thirst and polyuria. In Oct. 1968 she woke up with moderate hemiparesis on the right side. In addition to the hemiparesis, which was confirmed at the emergency ward, slight confusion, slow cerebration and slight tachycardia (100 beats/min) were observed at the physical examination. Laboratory tests showed consistent and significant hypercalcemia. The diagnosis of HPT was established, phosphate treatment was started and parathyroid exploration planned. The patient, however, died suddenly in the surgical ward, and in spite of immediate and vigorous efforts resuscitation failed. Autopsy revealed parathyroid adenoma and cerebral emboli.

Diagnostic criteria AD. Group I.

Case 5

Female, born 1895. At the age of 60 the patient suffered from hyperthyroidism, which was treated with I[131]. Since then she has been on small dose of desiccated thyroid. She has also had atrial fibrillation for 15 years, for which she has been digitalized. At the age of 70 the diagnosis of diabetes was made. Good control of this disease was obtained with an adequate diet. One year before admission she displayed symptoms of minor stroke. The day before admission, in Nov. 1969 the patient suddenly suffered from an attack of vertigo, with

Table VII. *Neurological and cardiovascular diagnoses and possible effect of operation on five patients with HPT and syncope*

Pat. no.	Neurological diagnosis	Cardiovascular diagnosis	Clinical effect of HPT operation	Postoperative observation period
13	Ischemia cerebri	Cardioarteriosclerosis, angina pectoris, hypertonia	Improvement	14 mo
14	Ischemia cerebri	Cardioarteriosclerosis, hypertonia	Improvement	7 mo.
15	Ischemia cerebri	Cardioarteriosclerosis, hypertonia	Improvement	10 mo.
16	Ischemia cerebri	Cardioarteriosclerosis, heart failure	Improvement	6 mo
17	Ischemia cerebri, Adams-Stokes attacks	Cardioarteriosclerosis, complete heart block	Improvement	3 y

attributed to cardiosclerosis. On 5 Oct. 1970 he was brought to hospital after an acute attack of syncope. He complained of loss of memory and general eakness. Physical examination showed essentially normal findings. Laboratory tests revealed hypercalcemia, and the diagnosis was confirmed and established. Operation, with exploration of the parathyroids, was performed. A parathyroid adenoma as found and removed. The postoperative course as undramatic.

Diagnostic criteria ABC. Group I.

Case 17

Male, born 1898. The patient had had symptoms of urolithiasis for several years. In Oct. 1966, in connec tion ith the passage of calculus through the left ureter a slight to moderate hypercalcemia was noted. HPT was suspected, but exploration was postponed for some time owing to cardiac insufficiency The following course as complicated by AV block, varying bet een grade I and III, which eventually resulted in several Adams-Stokes attacks, and treatment for several months with an internal pacemaker as initiated. Operation, with exploration of the parathyroids, was performed on 13 June 1967 An adenoma was found and removed. The postoperative course was undramatic Normocalcemia and sinus rhythm were restored postoperatively and cardiac pacing was judged to be unnecessary

Diagnostic criteria ABC. Group I.

DISCUSSION

In the present material the diagnosis of HPT in all the patients is fairly well established. Thus in the patients, with one exception (case 5) in sudden death occurred before the blood were made highly significant hypercalcemia was found on admission (Tables II and VI) Significant hypophosphatemia (not recorded in this paper) was also present in most of the cases.

In all the tients a morphological confirmation of the HPT diagnosis was also obtained by demonstration of parathyroid adenoma, hyperplasia or cancer at either operation or autopsy (Tables I II and VI)

The clinical manifestation of a major stroke in cases 1–12 was also very convincing Thus, symptoms and signs of hemiparesis were demonstrated in all these patients. In most of the patients a major stroke occurred immediately before admission. In some of the cases the attack of hemiparesis which caused the patient to be brought to the hospital was preceded by recent, transient attacks of hemipa esis

The tentative cerebrovascular diagnosis, based on history physical examination and, in some cases, on carotis angiography was considered to be cerebral embolism or cerebral thrombosis in most of the 12 patients. On the other hand none of these patients was suspected of having hemorrhagia cerebri. In some of the patients, however when carotis angiography failed to show obstruction of the cerebral vessels, the attack of hemiparesis was considered to be due to cerebral ischemia secondary to arteriosclerotic cerebrovascular disease. With regard to the possible occurrence of cardiovascular disease among the patients with hemiparesis, signs of mitral valvular disease with atrial fibrillation were found in one patient (case 1) A second patient (case 7) had a history of previous cardiac infarction and moderate hypertension and in a third (case 9) a transient sinus bradycardia was recorded. All the other patients were considered to be affected by cardioarteriosclerosis to about the same extent as would be expected for patients of similar age.

The sex and age distribution in the present material, of HPT associated with major stroke (Table I) differs very markedly from most other HPT materials. Firstly most of the patients in the present series were very old and secondly females were affected 3 times more often by HPT and major stroke than males. Recent studies based on extensive screening have indicated, however that the prevalence of HPT increases as a function of age very substantially in women after the menopause (3) This fact is reflected in the present material, in which most of the patients with HPT were found by screening for hypercalcemia. These patients had been admitted as emergency cases to St. Erik's Hospital or Serafimerlasarettet.

The exact nature of the cerebrovascular disturbances, observed in the particular cases of HPT in which the presenting symptom consisted of one or more attacks of syncope (cases 13–17), was essentially unknown. In some of the cases other transient neurological signs, e.g. alexia, aphasia apraxia and vertigo were also recorded. The most probable cause of these symptoms is, however cerebral ischemia, mainly due to cerebroarteriosclerosis. On the other hand it is also possible that transient cardiac arrhythmia, secondary to cardiovascular disease and hypercalcemia were the primary causes of the transient cerebral ischemia Thus one of the patients (case 17) had a third degree heart block with Adams-Stokes attacks, which necessitated pacemaker treatment

before the operation for HPT Postoperatively however sinus rhythm was restored and artificial pacing was no longer needed.

The present material, consisting of patients with HPT associated with major stroke or syncope, seems to be of considerable clinical interest in several respects. Firstly it is evident that the coexistence of HPT and major and minor stroke in the higher age groups is not unusual despite the very little attention paid to this in the literature.

It is not known at present whether or not the coexistence of HPT and stroke, reported in the present paper is only coincidental or actually reflects the existence of a physiological relation between high blood and cerebrospinal fluid concentrations of parathyroid hormone and calcium on the one hand, and changes in the excitability of central nervous tissues, in the clottability of blood in the cerebral vessels or in the development of cerebrovascular disease on the other However the fact that several patients showed a marked improvement in their neurological symptoms in connection with normalization of the parathyroid function seems to favor the view that HPT promotes the development of signs and symptoms of major and minor stroke.

The effects of parathormone and/or hypercalcemia on the vascular and cellular level are also illustrated by the more or less well known clinical connection between hypercalcemia in general, and HPT in particular and the following signs and symptoms: 1) acute psychosis, 2) proximal myopathy 3) venous thrombosis, 4) hypertension, 5) cardiac arrhythmia, 6) changes in EEG, EMG and ECG patterns. Therefore it seems reasonable to assume that also the development of signs of major and minor stroke reported in the present paper is partially caused and enhanced by the coexistent HPT

From our experience and for the above mentioned reasons it seems to be of considerable importance for the rehabilitation of patients with major stroke and coexistent HPT to restore normal parathyroid function as soon as possible In patients with signs indicative of minor stroke, operation of coexisting HPT at an early stage might even be of value for the prevention and postponement of the development of major stroke

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DEVELOPMENT OF A SECOND MONOCLONAL IMMUNOGLOBULIN G IN A PATIENT WITH LATE MANIFESTATION OF MYELOMA

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Abstract. In 1961 routine serum protein electrophoresis revealed the presence of monoclonal IgG in a patient who presented no clinical symptoms of myeloma. The paraprotein was of type G4/K. For five more years the patient remained in excellent general condition, and there was no change in the serum protein findings. In 1966 the occurrence of a second monoclonal γ-globulin band was noticed in serum protein electrophoresis and the coexistence of two IgG paraproteins of types G4/K and G1/L was established. The serum titer for Gm(1) was 1:8192 as compared to a titer of 1:64 in 1961. Titers for Gm(3) and Gm(5) had remained normal or low. In 1967 there was rapid development of polysymptomatic myeloma. Serum levels of myeloma protein increased continuously and, in late 1969, the patient's serum was found to contain 6 400 mg% of mono but G1/L type monoclonal IgG. There was no more evidence of G4/K paraprotein. The undiluted serum was negative for Gm(3) and Gm(5), but the Gm(1) titer had risen to 1:32 768. At that time the patient's clinical condition had become critical. She suffered from multiple pathological fractures, developed anemia and antibody deficiency syndrome, and expired in spring 1970. After a 5-year asymptomatic history of "benign" G4/K type paraproteinemia the occurrence of clinical myeloma together with a shift to G1/L type paraproteinemia is postulated to be a second disease due to proliferation of a second "malignant" plasma cell clone.

The occurrence of large quantities of immunoglobulin with characteristic electrophoretic and immunochemical homogeneity in serum (paraproteinemia) is most commonly recognized in patients with myeloma or Waldenström's macroglobulinemia. In some cases, however, paraproteinemia was established 6–17 years prior to the clinical onset of myeloma (6, 10, 11, 18, 22). Therefore it was concluded that developing myeloma can exist for many years as a pre- or oligosymptomatic period (11). During such a period paraproteinemia may be the only unequivocal indicator of the incipient malignant disease of immunologically competent cells, usually identifiable as plasma cells.

In the past ten years a total of over 300 observations have been published on patients with paraproteinemia who revealed no symptoms of myeloma or macroglobulinemia Waldenström during follow-up periods of up to ten years and more (2, 3, 5, 7, 12, 13, 14, 15, 16, 21, 25). Paraproteinemia has been found in association with a large variety of conditions as well as in clinically healthy individuals. Among a Swedish adult population of 6 995 persons, relatively low levels of paraproteins were found in as many as 60 healthy individuals (1). Designations such as "idiopathic paraproteinemia" (13, 14), "benign, essential monoclonal hypergammaglobulinemia" (22, 23) or "lanthanic dysimmunoglobulinemia" (25) have been proposed to characterize persisting paraproteinemia without development of clinical symptoms of myeloma, macroglobulinemia Waldenström, or other malignant lymphoreticular disorders.

As yet it has not been established whether or to what extent, "idiopathic" paraproteinemia is a potentially malignant condition (6, 7) or a "benign, essential" abnormality of the immunoglobulins (22). A case is reported in which multiple myeloma developed after a 5-year history of asymptomatic paraproteinemia. Simultaneously with the onset of clinical symptoms, a change of the antigenic subclasses could be established for both heavy and light chains of the patient's monoclonal IgG.

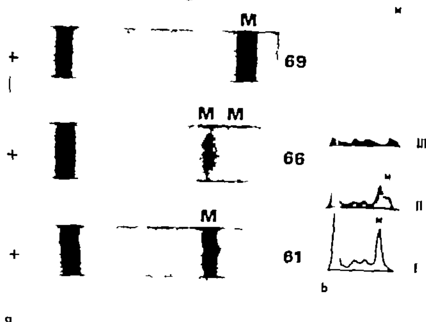


Fig 1 Electrophoretic findings in three selected serum samples from the patient.

(*a*) Protein separations on cellulose acetate strips.

(*b*) Corresponding electrophoretic diagrams. Serum samples drawn 3/21/61 (I) (asymptomatic paraproteinemia) and 6/23/66 (II) (onset of first clinical symptom) reveal M-components (G4/K type) in a fast γ-globulin position. In slow γ-globulin position M-components (G1/L type) appear in sera drawn 6/23/66 (II) and 11/26/69 (III) (clinical myeloma).

CASE REPORT

A 60-year-old white female, born in 1901. She was well developed and well nourished when seen for the first time at the Medical Department of the Tiefenau Hospital, Bern, Switzerland. Her personal history was not remarkable, except for rheumatic fever at the age of 14. In March 1961 she complained of nervousness, changing muscular tenderness, and insomnia. Results of the physical examination were within normal limits. Hb, WBC and differential WBC were normal, as were blood sedimentation rate (8/17 mm), serum chemistries, and urinanalysis. However, serum total protein was slightly elevated (8.44 g%) and electrophoresis revealed hypergammaglobulinemia with an M-component (2 100 mg%). Immunoelectrophoretic findings were diagnostic for IgG paraproteinemia. Neither generalized osteoporosis nor osteolytic lesions were noticeable on skeletal X-ray surveys, and possible idiopathic IgG paraproteinemia in a clinically healthy woman was suspected.

During the following five years the patient remained in excellent general condition. Repeated physical examinations and skeletal X-rays revealed no pertinent symptoms of myeloma. A series of electrophoretic serum protein studies confirmed the persistence of IgG paraproteinemia with little variation of the M-component concentration. Electrophoretically urine was always negative for Bence Jones protein. In 1963 the patient had a traffic accident resulting in a vertebral fracture of L1 which healed to completion. On this occasion iliac bone marrow aspiration was performed. Except for moderately increased, mature lymphocytes, the differential WBC was normal and not indicative of myeloma.

From spring to summer 1966 a definite change in the serum protein abnormalities was recorded electrophoretically. For the first time total protein (10.2 g%) and paraprotein concentration (3 400 mg%) increased significantly and the occurrence of a second M-component was noticed in the region of slowly migrating γ-globulins (Fig. 1 *a* and *b*). The immunoelectrophoretic pattern showed more complex irregularities of the IgG precipitation line than in previous years. Again, urine was negative for Bence Jones protein. In the early fall of 1966 the patient's general condition started to deteriorate. In the summer of 1967 extensive clinical manifestations of myeloma were evident, with acute onset of severe lumbar pain due to multiple vertebral fractures and osteolytic lesions. Iliac bone marrow examination revealed abundance of plasma cells which, in part, appeared in large clusters. Until spring 1970 the clinical course was characterized by skeletal generalization of the myelomatous disease. Brief remissions on intensive therapy with Alkeran® and Prednison were followed by new consecutive pathological fractures of the sternum, fractures of ribs, and progressive general deterioration. Anemia (5.6 g% Hb), excessively elevated blood sedimentation rate (134/160 mm) and hyperproteinemia up to 15 g% occurred with a paraprotein moiety of 50%. Serum viscosity was markedly increased. Electrophoretic diagrams revealed a single large M-component in the slow γ-region (Fig. 1 *a* and *b*). Correspondingly a typical deformation of the IgG pre-

cipitation line was found in immunoelectrophoresis (Fig. 2). In the terminal course, antibody deficiency syndrome developed with recurrent bacterial infections in the almost complete absence of immunoglobulins other than the monoclonal IgG. The patient died in May 1970. No autopsy was performed.

Parts of this case history have been published previously (14).

SERUM PROTEIN STUDIES

Between March 61 and March 70, 35 blood samples were drawn from the patient for serum protein studies. Electrophoresis was performed on each serum, and 25 of the samples were further analyzed immunoelectrophoretically. Three serum specimens drawn at different phases of the clinical course were selected for additional tests: March 21, 1961 (asymptomatic paraproteinemia), June 23, 1966 (onset of first clinical symptoms), and Nov. 26, 1969 (clinical myeloma).

Quantitative determinations of IgA and IgM were done on these three samples with a radial immunodiffusion technique (4). The sera were tested for Gm(1), Gm(3), Gm(5), and Inv(1) antigens (9). Light chains of the IgG paraproteins were typed with monospecific antisera from rabbit, and heavy chain subclasses were determined with specific antisera from sheep (anti-G1), monkey and pig (anti-G2, -G3, -G4) in double diffusion tests and immunoelectrophoresis (17). Tests for free light chains were performed with antisera specific to light chain hidden determinants.

Table I. *Concentrations of total protein and immunoglobulins in three selected serum samples from the patient*

	Serum drawn			
	3/21/61	6/23/66	11/26/69	Normal range
Total protein (g%)	8.44	10.2	1[illegible].25	6.5–8.[illegible]
IgG (mg%)	2 100	3 400	6 400	600–1 200
IgA (mg%)	143	70	0	90–350
IgM (mg%)	66	84	0	55–145
IgD[a]	0	0	0	
IgE[a]	0	0	0	
Free light chains	0	0	0	

[a] Double diffusion tests only

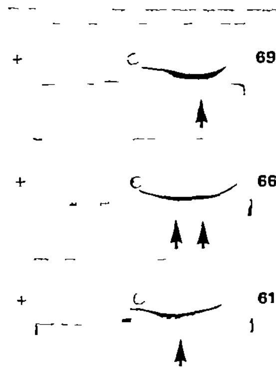


Fig. 2. Immunoelectrophoretic findings in three selected serum samples (same as in Fig. 1 a and b) from the patient.

All sera were tested in 1:5 dilutions with monospecific antiserum (rabbit) to human IgG. Well marked broadenings and indentations of the IgG precipitation lines ( ) characteristic of IgG paraproteinaemia occur corresponding to electrophoretic mobilities of the M-components.

RESULTS

Parallel electrophoretic runs of the three samples from the patient (Fig. 1 *a* and *b*) reveal the presence of an M-component in a fast γ-position (3/21/61), two M-components in fast and slow γ-positions, respectively (6/23/66), and a single M-component in a slow γ-position (11/26/69). In immunoelectrophoresis, IgG precipitation lines of the three sera present characteristic deformations (IgG paraproteinemia) corresponding to the above mentioned electrophoretic positions of the M-components (Fig. 2).

Estimates of the serum immunoglobulin levels are given in Table I. The sera were negative for Inv(1) and were not tested for Inv(3). The reciprocals of the titers for Gm antigens are listed in Table II.

Light chain typing and subclassification of the heavy chains of the IgG paraproteins in the three serum samples revealed one M-component type G4/K (serum drawn 3/21/61), two M-components of types G4/K and G1/L, respectively (serum drawn 6/23/66), and one M-component type G1/L (serum drawn 11/26/69). Immunoelectrophoretic results are presented in Figs. 3 and 4.

Table II. *Reciprocals of titers of Gm antigens in three selected serum samples from the patient*

All sera were Inv(−1)

	Serum drawn		
	3/21/61	6/23/66	11/26/69
Gm(1)	64	8 192	32 768
Gm(3)	128	256	Negative
Gm(5)	8	32	Negative

DISCUSSION

Results of the serum protein studies in our patient may be summarized as follows. In March 1961 when IgG paraproteinemia was first detected, an M-component of 2.1 g% was present, with electrophoretic mobility of a fast γ-globulin. Serum titers of the antigens Gm(1) and Gm(3) representing allotypes of the γ1 heavy chains of IgG as well as of Gm(5) which is a marker of the quantitatively predominant allotype of the γ3 heavy chains, were normal to low. Thus it could be concluded that IgG paraprotein in this serum (drawn 3/21/61) was not of the G1 subclass, nor was it likely to be of the G3 subclass, although the possibility of a Gm(1) myeloma protein was not excluded. Since G3 type paraproteins of the Gm(21) allotype are a rarity on the basis of the genetic markers the M-component was expected to be of either the G2 or the G4 variety (9 19). Determination of the heavy chain subclass with specific antisera was confirmative and revealed the paraprotein to be of type G4/K. Concentrations of IgA and IgM in this serum sample were normal. The negative tests for IgD and IgE are

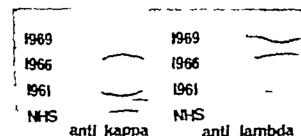


Fig. 3. Light chain typing of IgG paraproteins in three selected serum samples (same as in previous Figs.) from the patient by means of monospecific antisera (rabbit) to K and L type light chains in immunoelectrophoresis. Patient's sera and normal human serum (NHS) were used in 1 8 dilutions.

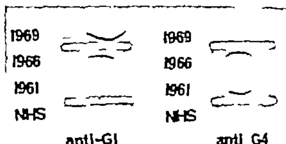


Fig. 4. Demonstration of heavy chain subclasses of IgG paraproteins in three selected serum samples (same as in previous Figs.) from the patient by means of subclass specific antisera to G1 (sheep) and to G4 (pig) in immunoelectrophoresis. *All sera were tested in 1 16 dilutions.* Corresponding tests for G2 and G3 subclasses are negative.

in accordance with frequent findings in normal individuals. Tests for free light chains were also negative.

In June 1966 the patient's serum was for the first time found to be markedly hyperproteinemic. In electrophoresis two neighbouring M-components were noticeable in the γ-region. Immunoelectrophoresis was indicative of IgG paraproteinemia. Tests for Gm antigens in this serum (6/23/66) revealed a startling increase in the titer of Gm(1). Although insufficient serum was available to permit preparatory isolation of the IgG paraproteins for separate Gm typings, the presence of Gm(1) allotype myeloma protein of the γ1 heavy chain subclass was postulated. Immunochemically two co-existing paraproteins were established, G4/K and G1/L. The serum level of IgA was moderately decreased, the IgM concentration was normal.

In Nov 1969 when the patient suffered from multiple myeloma, IgG paraproteinemia of only the G1/L variety was present. There was an almost complete deficiency of immunoglobulins other than monoclonal IgG.

Discovery of paraproteinemia in our patient was fortuitous, and it is unknown how long she might have had monoclonal hypergammaglobulinemia prior to the first serum protein study done in early 1961. Neither at that time nor during the next five years did she display clinical symptoms of myeloma. Serum concentrations of the M-component were moderate and remained practically stable through 1966. Her general condition was very good and it is evident that

during this 5-year period the patient belonged to the category of "idiopathic paraproteinemia" or "benign, essential monoclonal hypergammaglobulinemia". Between summer 66 and summer 67 there was rapid development of polysymptomatic myeloma and she survived only three more years (8) despite rigorous therapy. The sudden change in the clinical condition was accompanied by the occurrence of a second type of IgG paraprotein in rapidly increasing concentrations. At the same time the initially observed type of IgG paraprotein disappeared completely and there was a continuous decrease in the other immunoglobulins, too.

It seems clear that this patient with an initially asymptomatic paraproteinemia of type G4/K developed myeloma as a second disease leading to the proliferation of a second plasma cell clone which produced G1/L type paraprotein.

It has been suggested that single immunoglobulin heavy chains are the products of two separate structural genes (20). The same may be true for the synthesis of light chains. Because of the differences in both light chain types and heavy chain subclasses of the two consecutively occurring IgG paraproteins, secondary development of a competitive plasma cell clone is postulated in our patient rather than a mutation of the original strain. The results of this observation suggest that thorough study of the dynamics of monoclonal immunoglobulins in patients with idiopathic paraproteins as well as in cases of myeloma with an extended presymptomatic period is warranted.

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APLASTIC ANAEMIA

I. *Incidence and Aetiology*

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Abstract. Eighty patients with aplastic anaemia have been found among 1.2 mill. inhabitants in Sweden during the 5-year period 1964–68, corresponding to an incidence of 13/1 mill. and year. Only 13 patients (16%) were alive 3–7 years after the initial symptoms, most patients dying after a comparatively short time (< 2 y.). The aetiology in most patients remains unknown, more often so among older (81%) than younger (38%) patients.

Aplastic anaemia has been said to be "the most disabling, most frequently fatal and, next to agranulocytosis, the most frequently reported of adverse reactions to drugs" (9). An increase in the number of patients has been reported from Switzerland (6). Approximately 50% of the patients with aplastic anaemia are said to have been exposed to drugs or chemicals (9, 12, 16).

In order to find the incidence of aplastic anaemia in the community and to get a base line for an analysis of the drug-induced cases, we have studied all patients with aplastic anaemia hospitalized in one health care region of Sweden during a 5-year period (1964–68).

MATERIAL

In one of Sweden's seven health care regions (the Uppsala region—1.2 mill. inhabitants) all hospital diagnoses are recorded on a computer. We have collected all patients discharged with a diagnosis of aplastic anaemia (1955 ICD classification 292.40 and 292.50) during the 5-year period 1964–68. Under these headings were listed cases labelled as "aplastic anaemia" as well as pancytopenia. These diagnoses in most instances are used as synonyms without reference to the cellularity of the bone marrow. Through the courtesy of the head physicians of the various departments we have been permitted to study all the case records. A total of 102 patients (Table I) were found, of whom 22 were excluded for various reasons given in Table II.

RESULTS

Eighty patients were thus available for study. Thirteen could be regarded as accumulated from previous years, having a duration of disease of more than two years before 1964, the first year of our study. This leaves a fairly constant number of new cases each year, 10–15 in the Uppsala region, corresponding to 70–100 new cases in the whole of Sweden.

The age and sex distribution is given in Table III and the incidence (men and women together) in Table III and Fig. 1. There are exactly as many cases in men as in women. The overall incidence is low and constant up to the age of 50, whereafter it rises rapidly—80% of all the patients being ≥ 50 years old.

The aetiology in most patients remains obscure. There exists an age difference, however, inasmuch as among the younger patients (< 50 y.) only 38% are listed as of "unknown aetiology", whereas in the older group no less than 80% occur without any apparent reason (Table IV). The difference between these figures is highly significant (χ^2-test, $p < 0.001$).

Two of the patients suffered from epidemic hepatitis in direct connection with onset of aplastic anaemia. Two others had a probable viral upper respiratory infection. Drugs were probably responsible for ten cases (Table V) with seven deaths.

The mean haematological values on admission were: Hb 7.0 g/100 ml, WBC 2 400 and platelets 55 000. The initial symptom generally was fatigue. Although most patients also had leukopenia and thrombocytopenia, infections and/or bleeding

Table I. *Aplastic anaemia in the Uppsala health care region*

	Diagnoses recorded	Exclusions	No. of pats.
Accumulated cases	13	—	13
New cases			
1964	21	3	18
1965	20	7	13
1966	12	4	8
1967	17	4	13
1968	19	4	15
Total	102	22	80

symptoms were rare as a cause of admission (see Discussion).

The prognosis is bad. Most patients died within a short time (Table VI) 30% within 3 months, 61% within 2 years. At follow-up 3–7 years after the initial symptoms, only 13 patients (16%) were alive.

DISCUSSION

The number of new cases of aplastic anaemia is fairly constant 10–15 each year in the Uppsala region which, calculated for the whole of Sweden (8 mill.) would correspond to 70–100 new cases These figures are somewhat lower than agranulocytosis with 90–120 new cases each (15) and much lower than for thrombocytopenia with 450–500 cases each year (2) The incidence of the three haematological disorders,

Table II. *Exclusions—other anaemias incorrectly recorded as "aplastic"*

	Men	Women	Total
Thrombocytopenia	1	2	3
Anaemia secondary to malignant neoplasms	2	1	3
Slight pancytopenia (no aplasia)	1	2	3
Hypersplenism	1	1	2
Hepatic cirrhosis	—	2	2
Megaloblastic anaemia	—	1	1
Uraemia	1	—	1
Iron deficiency anaemia	—	1	1
Haemolytic anaemia	—	1	1
Acute infection	1	—	1
Patients belonging to other health regions	—	4	4
Total	7	15	22

Table III. *Aplastic anaemia in the Uppsala region 1964–68—age and sex distribution*

	No. of pats.				
Age (y)	Men	Women	Total in 5 y	Average per y	Incidence (per 100 000 and y)
0–4	—	2	2	0.4	0.4
5–14	2	2	4	0.8	0.5
15– 4	3	—	3	0.6	0.3
25–34	2	2	4	0.8	0.5
35–44	—	1	1	0.2	0.1
45–54	3	5	8	1.6	1.0
55–64	6	2	8	1.6	1.1
65–69	7	4	11	2.2	3.7
>70	17	22	39	7.8	7.4
Total	40	40	80	16.0	1.3
Whereof fatal	34	33	67	13.4	1.1

as well as the percentage of the drug-induced cases, etc. will be discussed in a separate report (5)

The overall incidence of aplastic anaemia in our material is 13/1 mill. the incidence of fatal cases 11 (Table III). The incidence increases with age (Fig. 1) Wallerstein et al. (14) found an occurrence of fatal cases of aplastic anaemia of 2/1 mill each year Considering the fact that both the calculations of Wallerstein et al. and ourselves have been performed on small materials (60 and 80 patients, respectively) and that our group has a slightly higher median age (69 against 62 y) and a somewhat higher proportion of cases without known aetiology (73 against 67%), we would assert that the two incidence figures agree remarkably well.

The age distribution is of special interest. The incidence is low and constant up to the age of 50, when it starts to rise. At high age it rises rapidly (Fig. 1) No less than 80% of all patients are above 50 years, 50% above 70. When at the same time it is possible to find that the cause of the aplastic anaemia among the younger patients (< 50 y) is known to a much larger extent than among the older ones (Table IV)—62% as compared to 19%—it seems reasonable to talk of two different groups of aplastic anaemia. One occurring at all ages, is a type in which drugs, acute infections etc. seem to play an important role as the aetiological factor the other—numeri-

Table IV *Aetiology of aplastic anaemia*

	< 50 y			> 50 y		
Drugs (see Table V)	2			2		
Congenital (Fanconi type)	3			—		
Malignant lymphoma	1			3		
Infectious hepatitis	1			1		
Acute infections	2			—		
Immunological mechanism	1	10		—	12	
Unknown aetiology		6	38%		52	81%
Total		16			64	

Table V *Drug-induced cases among the total material of aplastic anaemia (no. of deaths within parentheses)*

	No. of cases	
Definite		
Chloramphenicol	4	(2)
Phenylbutazone	1	(1)
Possible		
"Polypharmacy"	3	(2)
Oxyphenbutazone	1	(1)
Tolbutamide	1	(1)
Total	10	(7)

cally the largest group—is an aplastic anaemia of old age in which no exogenous toxic influences can be traced. One gets the impression that in these patients the bone marrow is the organ that gets "exhausted" first and that aplastic anaemia is the cause of death in the ageing patient instead, of e.g., general or cardiac atherosclerosis. Of course, it could well be that atherosclerosis of the bone marrow is the real villain. Knospe and Crosby (11) have recently put forward an interesting theory that aplastic anaemia is a disorder of bone marrow sinusoidal microcirculation rather than a stem cell failure.

Drugs were found as the aetiological factor in ten patients (12.5%) only one having been reported to the Swedish Adverse Drug Reaction Committee. Chloramphenicol (4 patients) remains the most commonly involved drug—with a high morbidity. The drug-induced cases will be discussed in detail in the second part of this paper together with the cases reported to the Swedish Adverse Drug Reaction Committee (3).

It is of special interest to find two patients who had aplastic anaemia shortly after an attack of infectious hepatitis. This connection has been much discussed in later years (summarized in (7))

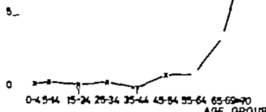


Fig. 1. Age-related incidence of aplastic anaemia in the Uppsala health care region, 1964–68.

and a number of individual cases have been published. Hilton (8) however was unable to find any history of preceding hepatitis in 183 cases of acquired aplastic anaemia.

The findings in our material are interesting enough to motivate a separate report (4) in which the patients will be discussed in detail. Nevertheless, our findings would correspond to an incidence of hepatitis as the cause of aplastic anaemia—or connected with the anaemia—of 2–3%. Furthermore in our material there were two additional patients in whom the records indicated acute, probably viral infections in direct connection with the onset of aplastic anaemia. A woman, aged 44 had "influenza" and one man, 31 had upper respiratory infection shortly before the onset of aplastic anaemia, which in both patients caused their death within a few months.

One woman had a complicated immunological disease. She had been thymectomized 13 years previously and later developed haemolytic anae-

Table VI *Course of aplastic anaemia in the Uppsala region*

		%	
Dead			
Within 0–3 mo	24	30	61
Within 4–12 mo.	12		
Within 1–2 y	13		
Later than 2 y	18		
Total	67	84	
Alive at follow-up	13	16	
	80	100	

mia which progressed to aplasia and caused her death at the age of 34. There are many connections between haemolytic and aplastic anaemias, as recently discussed (1). No other patient with a thymoma has been found in the material.

Four patients have been included in whom only the post-mortem examination showed that they suffered from malignant lymphoma. They had been ill with aplastic anaemia for a few months up to 3 years and it is impossible to decide afterwards whether the malignant lymphoma had been present all the time or followed on an initially present aplastic anaemia.

Most patients have been admitted because of fatigue. Regardless of the fact that most of them also had leukopenia and thrombocytopenia, those findings have not indicated hospitalization. This is probably due to definitions—if the patient has leukopenia, such as to cause infections, or thrombocytopenia causing bleedings, the case is listed as agranulocytosis or thrombocytopenia regardless of whether an anaemia co-exists. The cases registered as aplastic anaemia are those in which erythrocytopenia has been the dominating finding. The initial blood values found on admission are very similar to those reported by Lewis (12) and Wallerstein et al. (14).

The overall prognosis is bad and most of the tients died after a comparatively short time ble VI) 30% within 3 months. This agrees well with the findings in other similar materials (10 12). A follow-up study revealed that only 13 patients (16%) were alive 3–7 years after the initial symptoms (15%). Only one had a persistent anaemia 5 years later the others were in good condition with normal blood values. Lewis (12) found complete remissions in 10%.

The surviving group has a lower mean and median age (43 and 53 y respectively) than the total group, in which the corresponding figures are 61 and 69 years, respectively.

Among the patients who eventually die a few may be kept alive with blood transfusions for a remarkably long period (5–15 y). One patient, who had a complete remission after two years of treatment with testosterone, has been published elsewhere (13). It could be demonstrated that his remission was due to the testosterone medication as his blood values dropped when the androgen treatment was stopped and increased when the treatment was reinstituted. Most patients have been given blood transfusions, corticosteroids and testosterone preparations of various kinds. It has not been possible to evaluate the effect of therapy from the available records in a systematic way but the general impression is that treatment does not seem to influence the outcome to any measurable degree. This is in agreement with the findings of Lewis (12), who stated that in adults the prognosis and median survival time seemed little influenced by therapy.

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APLASTIC ANAEMIA

II. *Drug-induced Aplastic Anaemia*

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Abstract. Twenty-eight cases of drug-induced aplastic anaemia have been reported to the Swedish Adverse Drug Reaction Committee *during its first five years of existence*. This corresponds to an incidence of 0.7 cases in 1 mill. each year but seems to represent only one third of the occurring cases. The drugs most commonly involved are oxyphenbutazone (Tanderil®), chloramphenicol and phenylbutazone (Butazolidin®) in that order. The mortality is high, 66%.

Aplastic anaemia is the most devastating hematological complication of drug therapy with a very high mortality (50–80%). It has been stated that some unusual exposure to drugs or chemicals can be demonstrated in approximately half of all patients with aplastic anaemia (7, 12). This is not in accordance with our results in a preceding paper on aplastic anaemia (5), where it is shown that only 10 out of 80 patients (12.5%) had a drug-induced disease. The percentage of drug-induced cases, however, will vary with the way in which the material is collected. The present paper is a study of the cases of drug-induced aplastic anaemia, reported to the Swedish Adverse Drug Reaction Committee during its first 5 years.

MATERIAL

The Swedish Adverse Drug Reaction Committee was established in Oct. 1965. Up till Dec. 31, 1970, a total of 28 cases of aplastic anaemia had been reported, in which the Committee had judged the relation between the drug and the anaemia as probable.

RESULTS

The number of reported cases is constant during the period with an average of 5 reports each year.

The sex and age distribution of the patients is given in Table I, the age-related incidence in Table I and Fig. 1.

The offending drugs have been listed in Table II, which also gives the mortality. The total mortality was 19 patients (66%) with the highest figure in the chloramphenicol group (4 deaths out of 5 patients).

A calculation of the frequency with which cases are reported may be made from the figures given in the preceding paper (5). Five unreported cases were found in the Uppsala region during a 3-year period (1966–68). This would correspond to 33 unreported cases in the whole of Sweden during a period when 17 cases were reported. Thus there occurred 50 cases, out of which only one third (34%) were reported to the Committee.

DISCUSSION

There are two groups of drugs associated with bone marrow depression: 1) those that regularly produce bone marrow aplasia when given in large enough doses—this is the case, e.g. with drugs used in cancer chemotherapy and 2) those that cause bone marrow damage occasionally and in an unpredictable way, e.g. chloramphenicol and phenylbutazone (12). All cases caused by chemotherapeutic drugs have been excluded from this study—marrow aplasia in such cases should not be listed as a side-effect but rather as an exaggeration of the expected result.

Several reports are to be found giving the drugs most frequently causing aplastic anaemia (1, 3, 12). Most of the figures emanate from the AMA Registry on Blood Dyscrasias and are based on

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APLASTIC ANAEMIA

III. *Aplastic Anaemia and Infectious Hepatitis*

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Abstract. Two patients with fatal aplastic anaemia developing after infectious hepatitis have been found among 80 cases of aplastic anaemia during the 5-year period 1964–68. Approximately 900 cases of infectious hepatitis were registered during the same time. The findings would indicate that at least 2.5% of the aplastic anaemias are connected with infectious hepatitis and that the risk of developing aplastic anaemia after viral hepatitis is in the order of 0.1–0.2%. Two additional patients with the same combination of hepatitis and aplastic anaemia are described from the years following the original 5-year period. A survey of the literature is given.

During recent years a hitherto unexplained connection has been found between infectious hepatitis and aplastic anaemia. Leukopenia and/or thrombocytopenia have been known to accompany or follow viral hepatitis, but the first case of frank aplastic anaemia was described by Lorenz and Quaiser (11) in 1955. Today (Dec. 1971) at least 75 cases may be found in the literature and the *number rises every day. Hilton (7) however was unable to find any history of* preceding hepatitis in 183 cases of acquired aplastic anaemia collected over a 20-year period and concluded that infectious hepatitis must be an extremely rare cause of aplastic anaemia.

During a study of aplastic anaemia occurring within a well defined part of Sweden during a 5-year period (1964–68) we have found two cases of plastic anaemia after infectious hepatitis. This seemed interesting enough to warrant a publication, especially as our findings allow a calculation of the risk of developing aplastic anaemia after hepatitis from figures of the incidence of aplastic anaemia and hepatitis, respectively.

MATERIAL

Eighty cases of aplastic anaemia were found in one *health care region (1.2 mill. inhabitants)* in Sweden during 5-year period 1964–68 (5). In the same region during the same period 895 cases of infectious hepatitis are reported.

RESULTS

Two patients with infectious hepatitis immediately before the onset of aplastic anaemia were found. This corresponds to 2.5% of all patients with aplastic anaemia and 0.22% of all patients with infectious hepatitis.

Two other patients were reported to the Swedish Adverse Drug Reaction Committee in the years following the above mentioned 5-year period (1969 and 1970). They both had received drugs shortly before they fell ill with hepatitis, but will nevertheless be included in this report.

CASE REPORTS

Case 1

A 16-year-old boy, previously healthy, fell ill in Oct. 1967 with abdominal pain and became icteric a few days later. He was hospitalized on Oct. 20, 1967 as a case of infectious hepatitis. He had had no contact with drugs and had not received any injections. The course was uncomplicated, his SGOT rose to 330 U on Nov. 6 and gradually fell off. He was discharged in good condition on Dec. 7.

His Hb on admission was 14.1 g/100 ml and fell gradually during his stay in hospital to 11.3 on the day of discharge. On Jan. 2, 1968, it was 13.0 but on March 19 only 9.1. Further examination revealed WBC 1 100 and platelets 18 000. He was admitted to the Department of Medicine and vigorously but unsuccessfully treated for his aplastic anaemia, to which he succumbed on June –, 1968.

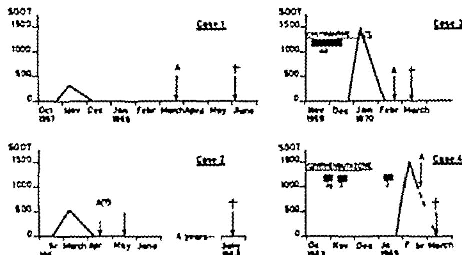


Fig. 1 Schematic description of the course in four patients with aplastic anaemia after infectious hepatitis. The time of hepatitis is indicated by the base of the triangle, the top of which indicates the time and the degree of maximal hepatic damage (SGOT U/ml). The onset of aplastic anaemia is indicated by the arrow under *A*, the time of death by the arrow under †

Case 2

A 74-year-old woman, who had previously suffered from various uncomplicated illnesses, but except for a moderate arterial hypertension and a leg ulcer had been healthy during the last years, was taken ill in the beginning of Feb. 1964 and became jaundiced on Feb. 16. On Feb. 22 she was admitted to hospital and treated until April 8 with a diagnosis of infectious hepatitis.

Her bilirubin rose to a maximum of 11.6 mg/100 ml, her SGOT values reached a maximum of 600 at the beginning of March. Her clinical course was uncomplicated and she was discharged in good condition. Her blood values remained normal during her stay in hospital.

Five weeks later she complained of fatigue and visited the hospital. She had started to find petechiae almost immediately after discharge from the hospital, but had not been "bothered" by them. An examination at this *time (May 17, 1964) revealed a profound anaemia (Hb 5.8 g/100 ml)* with leukopenia (2 000) and thrombocytopenia (32 000). She was admitted to the Department of Medicine, where she was given blood transfusions, corticosteroids and testosterone with no immediate effect. *Gradually however she improved and could be discharged* on July 20. With the help of blood transfusions she was kept alive until Nov. 1968 when she expired. At that time she had been given a total of 90 U blood during a period of 57 months.

Case 3

A 17-year-old girl, who in the beginning of Nov. 1969 visited a practising physician for a urinary tract infection. Bacteria in urinary sediment were found but no cultures were done. She was given chloramphenicol, 1 g daily for almost 40 days. She stopped the medication in the middle of Dec. On Dec. 22 she became jaundiced and was hospitalized a few days later. Bilirubin rose to 22.8 mg/100 ml, SGOT to 1 450 U early in Jan. 1970 but the values rapidly became normal and she was discharged after one month. Two weeks later a haemorrhagic diathesis was observed and she was readmitted to the hospital, this time with aplastic anaemia, Hb 8.8 g/100 ml, WBC 6 300 and platelets 17 000. The haematological values deteriorated rapidly and she died from septicaemia three weeks later.

Case 4

A 28-year-old man, previously healthy, was in late Oct. 1968 treated for prostatitis with penicillin and oxyphenbutazone. A second course of treatment was given a few weeks later this time with dimethylchlortetracycline and oxyphenbutazone. In Jan. 1969 he was again given oxyphenbutazone now for "low back pain". In all he took 3 × 30–90 tablets (0.1 g) oxyphenbutazone during *these three treatment periods. On Jan. 20 he was found* to be jaundiced and was admitted to hospital. His haematological values on admission were entirely normal with Hb 14.6 g/100 ml, WBC 3 600 and platelets 199 000.

His hepatitis, however, thought to be due to a toxic effect from oxyphenbutazone, ran a course typical of infectious hepatitis. On Feb. 7, 1969 his bilirubin reached a maximum of 21.6 mg%, SGOT 1 500. Already on Jan. 29 a liver biopsy had shown a picture typical of hepatitis. It was found impossible, however, to state whether the hepatitis was infectious or toxic. On Feb. 19 petechiae were noted and his haematological values fell rapidly. One week later the Hb was 8.7 g/100 ml, WBC 800 and platelets 14 000. In spite of vigorous therapy he died on March 11 from septicaemia and bleedings.

The clinical course of the four patients is summarized in Fig. 1. Information from the literature has been tabulated (Table I).

DISCUSSION

Today at least 75 cases of aplastic anaemia after infectious hepatitis may be found in the literature. Rosner (12) gave references for 55 cases—excluding four who had drug exposure· the following have appeared later (2, 3 4 6, 8, 9 16) and the number of known cases seems to rise every day There can hardly be any doubt at this time that a causal relationship exists between hepatitis and bone marrow aplasia. However nobody has been able to explain the pathogenesis. There is virtually nothing to support the idea of an autoimmune mechanism, although antibodies to leukocytes and platelets have been described in a few instances (14) An indirect toxic effect, implying that a damaged liver would not be able to detoxify a noxious agent, seems unlikely as most patients have an entirely adequate liver function at the time of onset of aplasia (10, 13) Until further knowledge is gained, one has to assume that the hepatitis virus acts directly on the bone marrow stem cells themselves. Rubin et al. (13) suggested a chromosomal damage to the haemato poietic system with subsequent stem cell failure. It is quite likely that we shall not learn more about the pathogenesis until the hepatitis virus has been isolated and cultivated—an event that has been keenly looked forward to during several decades.

Table I. *Information from the literature regarding infectious hepatitis and aplastic anaemia*

	n^a	
Sex	46	♂ 65 % ♀ 35 %
Age (y.)	46	Mean 20 Median 17 Range 2–74
Maximum SGOT values (U/ml)	23	Mean 1 130 Median 1 100 Range 50–3 600
Interval between onset of hepatitis and aplasia	40	Mean 6 weeks Median 4 weeks Range 0–36 weeks
	2	5 y 8 y
Outcome	46	Dead 78 % Alive 22 %

^{a} Indicates the number of patients for whom specific information is available.

A genetically determined hypersensitivity of the bone marrow has been suggested by Boga and Szembre (4), who reported aplasia in two sisters, 5 and 8 years of age, after infectious hepatitis. These intervals, however are the longest on record and long enough to make it difficult to ascertain the degree of relationship between hepatitis and aplasia.

We have tabulated the information from the literature regarding sex, age extent of liver damage and interval between onset of hepatitis and aplasia in the cases where such information has been given (Table I) The proportion of female cases has increased somewhat since the first reports. Available data, however indicate that 65% of the patients are male. It is evident that children and young people are more vulnerable than adults. Only a few cases are found in patients above the age of 25 (8 of 46 = 17%). No relation between the degree of hepatic involvement and the interval to onset of aplasia, nor to the final outcome, seems to exist. In a few patients aplasia appears already during hepatitis, in most it becomes apparent *after* the hepatitis has subsided but within a short interval—90% of the patients have an interval shorter than 3 months between the onset of hepatitis and the onset of aplasia.

In a total of six cases the patients have been given drugs known to be potentially bone marrow toxic before the onset of hepatitis. Five patients have received chloramphenicol and one oxyphenbutazone (1 8, 13 present authors) In all these six patients the liver disease has been from all aspects a typical infectious hepatitis. In a few cases liver biopsies have been performed, but it was not possible to distinguish between toxic and infectious hepatitis. If these cases are viewed in relation to all the others that have not received any drugs, it is our opinion that the medication in these instances has been coincidental and with little—if any—importance for the development of hepatitis and aplasia. On the other hand it has been shown that, e.g., chloramphenicol bone marrow toxicity seems to be increased in patients with hepatic or renal insufficiency (15) The patients discussed here, however had normal liver function when they were given the drug in question.

It is of interest that our data permit a calculation of the risk of developing aplasia after hepa-

titis. Two patients with an aetiology of hepatitis among 80 cases of aplastic anaemia means that at least 2.5% were connected with viral hepatitis. In our material another two patients with acute virus infections preceding the bone marrow aplasia were found. A woman aged 44 had "influenza" and a man, aged 31 had upper respiratory infection shortly before the onset of aplastic anaemia. In both cases initial normal WBCs exclude the possibility that their infection was secondary to aplasia and leukopenia. If these two patients are included, a total of four patients, i.e. 5% had a viral aetiology

During the years 1961–68 895 cases of infectious hepatitis were registered, in two of whom aplastic anaemia followed, i.e. 0.22% of the hepatitis cases were followed by bone marrow aplasia.

In the two years (1969 and 1970) following the original study of aplastic anaemia (5) one case of aplastic anaemia after hepatitis has been reported each year to the Swedish Adverse Drug Reaction Committee (cases 3 and 4). The total number of hepatitis patients in Sweden would be in the order of 1 200 each year which would give a risk of bone marrow aplasia of 0 08% Considering that this calculation is made only on the two patients who were reported as suspect drug reactions and that other cases of virus- [illegible] aplastic anaemia might have occurred, the [illegible]k figures are similar and would give an estimated risk of aplastic anaemia after infectious hepatitis in the order of 0 1–0.[illegible] %

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DRUG INDUCED AGRANULOCYTOSIS, WITH SPECIAL REFERENCE TO AMINOPHENAZONE

IV *Children*

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Abstract. Thirteen cases of agranulocytosis in children under 15 years have been diagnosed in Finland during 1966–67. This is an incidence of 1:185 000. In children under 5 years the incidence was 1:86 000. These frequencies correspond well with those for adults in Finland, but remarkably exceed estimates accepted in other countries. Twelve episodes occurring in ten patients were analysed in detail. The use of drugs suspected of causing agranulocytosis was found in ten episodes, aminophenazone and sulfonamides being the most common. The fatality rate was surprisingly high (50%), probably caused, at least in some cases, by administration of the causative drug after symptoms of agranulocytosis had appeared. Our results indicate that drug-induced agranulocytosis is as common among children as among adults and, therefore, that the same or more caution should be applied to the administration of these suspected drugs to children as to adults.

The occurrence of agranulocytosis among children and adolescents is very low. Wintrobe, in 1967 (7), *found in the literature only nine sub*stantiated reports of this disorder in children. In several studies (1, 2, 6) aminophenazone (Pharmacopoea Nordica; aminopyrine, United States Pharmacopoea) has been proved to be one of the most common causative agents of agranulocytosis. Therefore its use is quite limited in many countries. By contrast, in Finland aminophenazone in the form of suppositories is still sold over the-counter and is used frequently as an antipyretic for children.

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PATIENTS AND METHODS

This material was collected from the Department of Pediatrics, University of Helsinki, for the period 1954–70. It has been analysed in detail, particularly concerning the use of drugs, and all available information on drugs used during the 3-week period prior to the onset of symptoms of agranulocytosis has been recorded.

In addition, all cases of agranulocytosis occurring in 1966–67 were collected from all pediatric hospitals and departments in Finland. Only children under 16 years are admitted to pediatric hospitals in Finland.

Reported cases fulfil the following criteria: 1) typical clinical picture of severe illness and fever, 2) granulocytes less than 500/mm³, 3) bone marrow and peripheral blood showed no evidence of other blood disorders, and 4) erythropoiesis and thrombopoiesis were substantially normal. Cases, in which agents regularly causing bone marrow depression had been utilized (such as X-ray or cytostatic drugs), were excluded.

The frequency of agranulocytosis was calculated from information on the under 15- and the under 5-year-old populations of Finland and also on the sales of aminophenazone.

RESULTS

Thirteen cases of agranulocytosis were found during 1966–67 in all Finnish pediatric hospitals and departments, making an average of 6.5 cases/year.

The under 15-year-old population of Finland at the end of 1967 was 1 207 000. The incidence of agranulocytosis was 1:185 000 in this population. Of the ten cases collected from the Department of Pediatrics, University of Helsinki (Table I), seven occurred in children under 5 years. If this trend holds throughout the country

Table I. *Data on child patients with agranulocytosis*

Year	No. of episodes	No. of pats.	Sex	Age (y./mo.)	Granulocytes (no./mm³)	Drugs taken 3 weeks prior to onset of agranulocytosis	Fate
1957	1	1	♂	4/6	0	Sulphisodimidine, chloramphenicol, 3 different penicillins	Survived
1958	[illegible]	1	♂	3/2	0	Acetylsalicylic acid, unidentified cough medicine	Survived
1958	3	2	♀	2/2	30	Promethazine diphenhydramine, histamine-azoprotein complex, ammonium chloride, chloroform, menthol, acetylsalicylic acid	Survived
1960	4	3	♂	2/0	160	4 different sulfonamides, 2 different barbiturates, atropine histamine-azoprotein complex phenoxymethylpenicillin, hydroxyzine, tetracycline, aminophenazone, papaverine, acetylsalicylic acid	Survived
1961	5	4	♀	1/7	40	Acetylsalicylic acid, procain penicillin, aminophenazone	Died
1961	6	5	♂	0/2	40	Chloramphenicol	Died
1961	7	6	♂	2/2	0	Pentobarbital, aminophenazone, tetracycline acetyloleandomycine	Survived
1962	8	7	♀	1/11	0	Aminophenazone, codeine, diallylbarbituric acid	Died
1965	9	8	♀	6/10	0	Aminophenazone	Died
1965	10	9	♀	11/11	20	Penicillin, streptomycin, chloramphenicol, codeine, ephedrine, aminophenazone, diallylbarbituric acid	Survived
1966	11	9	♀	12/7	0	Phenoxymethylpenicillin, sulphasomidine	Died
1969	12	10	♂	10/11	290	Tetracycline, codeine, ephedrine	Survived

there is not reason to think it should not, number of cases of agranulocytosis in children under 5 years would be 4.5/year. The under 5-year-old population of Finland at the end of 1967 was 387 000. Thus, the estimated yearly incidence of agranulocytosis was 1 86 000 in this population.

In 1967 about 160 000 packages containing 5 aminophenazone suppositories and 80 000 packages containing 10 suppositories were sold in Finland. If we assume a package of 5 suppositories to be one course and that of 10 to be two courses, then a total of 320 000 courses of aminophenazone were sold in Finland during 1967. Since practically all aminophenazone suppositories are given to children, we may postulate an incidence of 6.5/320 000 courses or about one case of agranulocytosis/50 000 courses. This calculation, naturally is very approximate.

The cases of agranulocytosis collected from the Department of Pediatrics, University of Helsinki, are presented in Table I. The age and sex of the patients, the lowest number of granulocytes, the drugs used during three weeks prior to the appearance of symptoms of agranulocytosis, and the fate of the patients are noted in the Table.

Altogether 12 episodes of agranulocytosis in 10 patients were found. The male/female ratio was 1 1. The mean age of the patients was 5 years 2 months (range 8 weeks – 12 years 7 months). Five of ten patients (50%) died. The outcome does not seem to depend on the number of granulocytes or the kind or amount of the suspected aetiological agent administered before the symptoms of agranulocytosis appeared.

As seen in Table I many different drugs had been used in most cases. Patient 8 (episode 9) received only aminophenazone and, on the basis of this information aminophenazone may be considered a strongly possible causative agent. When analysing the possible causative agents in other cases, we find that aminophenazone was

administered in five cases (episodes 1 4 5 7 8 and 10) sulphisomidine in two (episodes 1 and 11) and chloramphenicol in one (episode 6). In episode 3 several drugs were given. None of these is known as a major causative agent of agranulocytosis. However it is known that phenothiazides, usually only after prolonged use, may cause agranulocytosis (5) A member of the phenothiazide group promethazine was administered to this patient for 6 months before the appearance of agranulocytosis. In two episodes (nos. 2 and 12) no agent known to cause agranulocytosis has been registered. Since the drug histories in both these cases were rather incomplete, the possible use of some agranulocytosis-causing agent cannot be excluded.

The appearance of the histamine-azoprotein complex (Lertigon®) in two cases is interesting, although its exact relationship to agranulocytosis remains uncertain.

The indication for the medication, which may have caused the agranulocytosis, was respiratory tract infection in seven episodes, allergic eczema in one, and urinary tract infection in two.

DISCUSSION

The incidence of agranulocytosis among children has been considered to be very low (6, 7) In the present material, however the estimated frequency is 1 185 000 in the population under 15 years and 1 86 000 in the population under 5 years. The total number of cases in this study is larger than in previous pediatric studies. In our earlier work (3) using the same criteria of evaluation as here for calculating the frequency of agranulocytosis, we obtained the frequency 1 100 000 among adults. Thus, the difference in frequencies between adults and children is not striking as suggested earlier but is, in fact, nonexistent. We shall discuss later the question whether this relatively high incidence concerns only Finland.

In the information on adult and child cases, the only clear-cut difference is the absence of female predominance in the latter group. In Finland the male/female ratio of the occurrence of agranulocytosis among adults is between 1 7 and 1 11 (3) but 1 1 among children. Whether this illustrates, on the one hand, a real sensitivity of adult women to agranulocytosis or on the other a consequence of the more common use of drugs among adult women is unclear but the latter possibility seems more likely

In this study the strong association between the use of drugs known to cause agranulocytosis and the occurrence of agranulocytosis supports the view presented by us earlier (3) that idiopathic agranulocytosis is a very rare disease if it exists at all. The causative agent may not necessarily be a drug but some other non-physiological compound in our environment. However in the light of this and our earlier study concerning adults (3) it seems that drugs are by far the most common causative agents of agranulocytosis in a modern community

Among children and adults the frequencies of association of a specific drug with agranulocytosis seem to be very similar (3). Aminophenazone is the most common associated drug, sulfonamides the second and phenothiazides probably the third. The association of these drugs with agranulocytosis has been convincingly proven earlier (7) It is very unlikely that this association is just a coincidence or a consequence of the ery common use of these drugs as compared with other drugs.

The high fatality rate in children (50%) which did not show any decreasing trend during the observation period, is surprising because the fatality rate of adults with agranulocytosis in Finland has decreased prominently—from 83% in 1950–55 to 13% in 1960–68 (3) When analysing the fatal cases of adults after 1960, we found that the administration of the drug possibly causing agranulocytosis after the beginning of symptoms was related positively to the death in almost every case (4) The same may be true in the pediatric material. For example, case 7 received 6 suppositories and 1/2 tablet of aminophenazone in the hospital when the low leukocyte count had been recorded. In the other fatal cases the condition of the children was already ery poor on admission. However the administration of the suspected drug was probably continued during the time when the symptoms of agranulocytosis existed. In most cases the indication for the use of the drug was a febrile condition and, therefore it is not easy to differentiate the symptoms of agranulocytosis from the symptoms of the original disease.

One interesting finding in our study is the relatively high incidence of agranulocytosis among

children in Finland. Whether the difference between Finland and other countries in this respect is true or only caused by lack of observations in other countries is as yet unsolved. However considering the lack of thoroughness of disease registration system in many countries, the first possibility seems more probable.

In Finland aminophenazone is still sold over the-counter and a great deal is used as an antipyretic for children. In some other countries (e g. USA, England, Sweden, Denmark and Norway) the drug is under prescription and, at least in England and Sweden medical or pharmaceutical societies have warned against its use. It would appear to be rarely used in those countries, although we do not have any exact figures concerning its sale or usage. Our study suggests that the main reason for the relatively high incidence of agranulocytosis in children in Finland is the extensive use of aminophenazone

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THE LIPOPROTEIN PATTERN IN A DANISH FAMILY

Children and Adolescents

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The so-called "normal values" may not necessarily be desirable values.

Henry (1964)

Abstract. The lipoprotein pattern in 32 children and adolescents, aged ..–25 years, of a family having primary hyperlipoproteinaemia (Hlp), was studied on the basis of clinical investigation and the criteria of Fredrickson et al. for biochemical studies of lipoproteins. The following distribution was found: type II 2 cases, type IV 2 cases, and 28 family members were assessed as normal. In spite of the biochemical findings in the children and adolescents having types II and IV pattern, none of them had clinical symptoms characteristic of Hlp. Lastly the authors discuss the implications of the biochemical findings in the 28 children and adolescents who did indeed show a normal lipoprotein pattern, assessed by the reference criteria for adults, but in whom some values of the studied variables are at the upper limit of the reference range.

In a previous paper (22) we reported the results of studying the lipoprotein pattern in the adult members of a Danish family (13 siblings + the father). Below we shall report our continued studies, this time dealing with the children of the former subjects.

METHODS

The investigation, carried out in the autumn of 1970, comprised 32 children and adolescents (generation III), as assessed at the same time the spouses of the 13 siblings, partly to rule out causes of secondary hyperlipoproteinaemia (Hlp) and partly to obtain information concerning the occurrence of possible genetic pattern through the lipoprotein types of the parents and children (5, 11).

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Out of the 32 children 14 were females and 18 males. Fifteen were in the age range ..–14 years and 17 aged 15–25, the age of 15 being taken to be the limit between child and adolescent. Twelve families had 1–7 children, while one couple had none.

All the subjects were investigated on an outpatient basis in the Central Laboratory. The investigation included complete history, physical examination with measurement of height, weight, and BP, ECG, and ophthalmoscopy. All the subjects were closely questioned about their use of drugs (in particular hormone preparations), smoking, and drinking habits.

The investigation was performed at 9 a.m. after the subjects had fasted from 6 p.m. on the preceding day. None had been on dietetic treatment.

All the lipid analyses were commenced immediately and with the exception of serum lipid electrophoresis the analysis of all the samples was completed on the same day.

Samples for immunoelectrophoresis were sent by express mail to H. G. Nielsen, Meda-Lab, Copenhagen, and this analysis was started about 14 hours after the sampling.

All sera, which on the basis of total cholesterol (TC), triglyceride (TG) and lipoprotein electrophoresis were primarily interpreted as types III or IV, were later forwarded for ultracentrifugation to K. Erdal, Laboratory of the Carlsberg Breweries, Copenhagen.

Serum

Determinations of total lipid (TL), TC, TG, lipoprotein electrophoresis, and Laurell immunoelectrophoresis were carried out by the methods described previously (22). Total β-lipoprotein (TbL) was determined by the method of Burstein and Samaille (2).

RESULTS

The following values were normal in all subjects: Hb, ESR, Hct, PBI, T₃ resin test, GOT, alkaline phosphatase, thymol turbidity, serum paper protein electrophoresis, bilirubin and blood glucose (overnight fasting). Urine microscopy and Hema-Combistix® were also normal.

Table I *Biochemical features in 32 children from a family with primary hyperlipoproteinaemia*
TL, TC, TG TbL, type cf Table II

Case no.	Age (y)	Sex	TL	TC	TG	TbL	Serum	Type[b]
III:01 1	25	♀	870	230	68	9	Clear	N/N
01.2	17	♀	710	184	67	9	Clear	N/N
01.3	7	♂	938	275	75	13	Clear	II/II
III:02 1	25	♂	1 065	187	386	20	Clear/cloudy	IV/IV
02.2	24	♂	780	223	64	12	Clear	N/N
02.3	22	♂	762	262	67	14	Clear	II/II
02:4	17	♂	605	163	48	7	Clear	N/N
02.5	14	♂	572	144	101	7	Clear	N/N
02:6	13	♂	692	188	130[a]	11	Clear	N/N
02.7	11	♀	804[a]	199[a]	97	11	Clear	N/N
III:03 1	18	♀	718	218	65	5	Clear	N/N
03.2	15	♂	722	168	127[a]	5	Clear	N/N
03.3	12	♀	818[a]	198	121	8	Clear	N/N
III:04 1	19	♀	865	221	124	12	Clear	N/N
04:2	15	♂	660	165	79	7	Clear	N/N
III:05 1	22		725	189	88	9	Clear	N/N
05.2	20	♂	694	191	82	9	Clear	N/N
05.3	18	♀	575	165	59	6	Clear	N/N
05:4	15	♂	668	185	39	6	Clear	N/N
III:06 1	14	♀	668	173	107[a]	8	Clear	N/N
06.2	3	♀	800[a]	192[a]	47	7	Clear	N/N
III:08 1	15	♂	570	173	46	8	Clear	N/N
08:2	12	♀	830[a]	235[a]	67	7	Clear	N/N
08.3	5	♂	585	192	72	9	Clear	N/N
III:09 1	17	♂	608	178	70	6	Clear	N/N
09.2	16	♀	770	220[a]	78	10	Clear	N/N
1	5	♂	730	218[a]	60	8	Clear	N/N
10:2	2	♀	705	178	105[a]	10	Clear	N/N
III 11 1	5	♂	700	187	68	3	Clear	N/N
III:12.1	8		665	188	42	7	Clear	N/N
12.2	4	♂	715	177	102[a]	10	Clear	IV/N
III 13 1	7	♀	764	177	58	9	Clear	N/N

[a] "At risk" persons? (see text).
[b] N = normal.

The results of the biochemical studies are listed in Tables I and II.

In the physical examination as well as in the determination of height, weight, BP and in ECG and ophthalmoscopy 30 of the subjects were found to be somatically normal, a deviation in b.wt. of ±10% (16) being considered within the range of normal.

None of the 32 children or adolescents had any kind of past or present xanthomatosis.

In family II:01 with three children one was severely mentally defective (cause unknown) and another had slight intellectual impairment presumably due to Little's disease. The serum amino acid pattern (10) was studied in these three siblings and their parents and proved normal in all. The mentally defective child (III:01.3) had shown a very pronounced β-band in the electrophoresis. His lipid values were normal assessed by adult standards, but must be considered elevated in view of his age.

In another family (II.13) one child had received digitalis during infancy because of congenital heart disease (endocardial fibroelastosis?) but at the time of the study he was fit, without any medication.

A 25-year-old clerk (III:02:1) was 40% over weight, but in good health, and the physical examination showed no abnormality BP 135/90 and ECG showed flattened T waves in all leads.

Table II. *Biochemical and clinical features in five relatives with hyperlipoproteinaemia*

Case no.	Sex	Age (y)	TL	TC	TG	TbL	Type[a]	± %	Comment
II:02:b	♀	45	1 040	41	230	21	IV/IV	+65	Xanthelasmata
II:09:b	♂	40	944	268	106	16	II/II	+ 5	
II.11:b	♀	29	1 065	240	284	20	IV/—	+30	Oral contraception
II:12 b	♂	31	993	254	187	19	IV/N	+ 5	
II.13:b	♂	33	1 220	252	320	22	IV/IV	+35	BP 180/100, angiopathia retinalis, incomplete RBBB

Lipoprotein electrophoresis/immunoelectrophoresis.
Deviation from normal weight (16).
Reference range after fasting for 14 hours. TL <1 000 mg/100 ml. TC 140–300 mg/100 ml. Conversion factor to mmol/l 259 10⁻⁴ TG 74–172 mg/100 ml. Conversion factor to mmol/l 114 10⁻⁴ TbL 4–10 U

(Lipid values listed in Table I.) His brother (III:02:2) was a gardener and 15% overweight. BP 145/90 and ECG showed splitting of the QRS complex. Their third brother (III.02:3) a labourer was found to be physically normal. By the two electrophoretic methods he was an evident type II (Table I).

In child III. 12.2 there was an extremely distinct pre-β-band which gave rise to a suspicion of type IV Immunoelectrophoresis showed normal appearances, and so did ultracentrifugation. However the TG value appeared to be somewhat higher than the average for children of the same age of this family A similar discrepancy was found in his father (II.12:b), whose lipid values are shown in Table II. In the other subjects there was complete agreement between paper and immunoelectrophoresis.

None of the children or adolescents showed any signs of vascular disease.

Lastly it may be mentioned that in this kindred there were no cases of abortion, premature delivery or deaths of infants or young children.

Among the 13 spouses five had Hlp (Table II). Six of the spouses were overweight (15–65%), but only three had elevated lipid values. Three had corneal arcus, one xanthelasmata, and one elevated BP with ECG and retinal changes. However none of these spouses exhibited symptoms of arteriosclerotic vascular disease.

Table I gives the biochemical *results for* all the children and adolescents. An *a* indicates children who were normal but in whom the TL, TC and/or TG values were at the limit of normal in relation to age and adult values, and who should be kept under close observation in the future, considering the type found in the parents.

Fig. 1 *a–f* illustrates the pedigrees for the six families in which one or more children showed a type differing from the parents' or in which both parents could be typed but in which the findings were still normal in the child(ren). It was not possible to carry out a genetic evaluation, as we do not know the lipid type of the ancestress

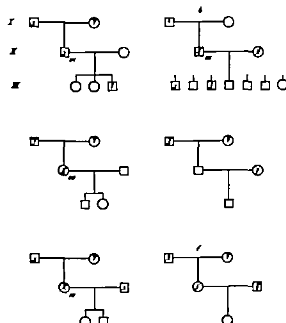


Fig. 1 a–f. Pedigrees showing the type of Hlp in six families in which either one or both parents and/or one or more children have been typed. □, normal ♂ ○ normal ♀. Roman numerals denote lipoprotein types according to Fredrickson et al. (5).
Concerning „normal" children cf. text and Table I.

Table III. *Survey of some reference ranges for lipid analyses (mg/100 ml) in children and adolescents*

Authors	Age (y)	Sex	TL	TC	TG
Kornerup (9)	1 14		820±158	209±40	
Rafstedt (19)	2–14	♀♂	490–1 090	158–242	
Josephson & Gyllenswärd (8)	6			222 (adult 238)	
Joosten et al. (7)	13–19	♂ ♀		180 (mean) 199 (mean)	
Stanbury et al. (21)	1–19	♀♂		115–240	25–150
Fredrickson et al. (5)	0–19			177±34	61±34
Lloyd (12)				126–230	40–125
Matthews (15)	3–28 (56) 13–17		500–1 000	150–250 176 (mean)	37 137
Fyfe et al. (6)				125–250	40–125

(generation I) but we have learnt that she had a severe arteriosclerotic disease (22) and that her sister had type II Hlp.

DISCUSSION

This is a report on the results of a lipid study in 32 children and adolescents of a family in which at least one of the parents had been found to have primary Hlp. Similar results have been published through many years, but in most of these previous studies (7 8 9 19)—those prior to the early 60's—there has not been a simultaneous determination of the TG only of TL, TC, cholesterol esters, phospholipid, etc. However a direct comparison is not always possible because of different analytical methods (1 5 23)

By far the great majority of investigators have been concerned with the problem of the lipid pattern (Hlp) in relation to coronary disease, other occlusive arterial disease diabetes mellitus, obesity and xanthomatosis per se, so that they have rarely reported a reference material of TL and TC in persons under 20 years of age (1 4 6, 17)

The interest in TG in recent years was aroused by the typing of Hlp suggested by Fredrickson et al. in 1967 (5) since by this method typing is easily done, not only in adults but also in children, on the basis of TC, TG and lipoprotein electrophoresis (13).

We also determined TL, since in our experience a value exceeding 1 000 mg/100 ml is abnormal—at any rate in adults—and should be followed up by electrophoresis. Polano et al. (18) are of the same opinion setting the limit at ≥ 1 100 mg/100 ml.

Assessment of the lipid pattern in children is difficult, as there have been but a few studies supplying a reference material for TL, TC, as well as TG in the age group 0–15 years (20).

On the basis of the literature we have tried to list in Table III publications stating reference ranges for either TL, TC or TG laying stress on the representation of children and adolescents in the materials.

Rafstedt (19) found greatly increasing TC values during the first days of life and in the age range 2–14 years values corresponding to the reference range for adults. Josephson and Gyllenswärd (8) found, from the 2nd to the 6th year of life, a TC value which was steadily increasing up to the adult values.

Pantelakis et al. (17) reported the determination of the lipid pattern in various Sf classes, including normal, non-diabetic children. A number of clinical studies make no mention of reference ranges for TL, TC, TG and in others it is seldom sufficiently clear how the authors have arrived at the values. In the case of children this may be due, partly to the slight variation in the lipid pattern before puberty and partly but mainly to the difficulty in fixing the upper limit of the reference ranges for healthy children. This applies quite particularly to TG (13 14).

So far we have not been able to set up a reference material, but we have used the values listed in Table II, fully aware of the problem, also pointed out by Carlson (3) that a child may show a normal adult" pattern. Most of the children in our material exhibited a normal lipid pattern, but a few had values so high that continued follow-up may perhaps soon reveal abnormalities.

According to Pantelakis et al. (17) a pre-β band is not normally found in children, and Levy and Fredrickson (11) found type IV to be extremely uncommon in children. However Wolff (24) has treated a child with a type IV Hlp.

Our subject III.12.2 had a distinct pre β-band, but a normal TG value. This has been reported in adults (4 5) as a normal phenomenon. Moreover a pre-β-band may be observed following a carbohydrate loading (20) and in diabetic children (17) We are unable to advance any explanation of this band in our cases, and we feel that by our investigations we have excluded diabetes.

An inspection of the types in the six small pedigrees (Fig. 1) shows no definite genetic pattern, but on the basis of the criteria set up concerning hetero- and homozygosity for type *II* (4 12, 15), it is perhaps permissible to draw a parallel and call III 12.1 and III.12:2 homozygotic.

The earliest age at which Hlp may be demonstrated has not as yet been determined for the various types of lipoproteins (13) but in families with early occlusive/ischaemic vascular disease *the children should be investigated at an early* age and treatment should be started if Hlp can be demonstrated (4 11 13 15 24). This view is our motive for performing the present study and publishing the results at the present time, because we have not been able to find a similar study of children in the literature.

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SIGNIFICANCE OF GLUCOSE LOAD IN ORAL GLUCOSE TOLERANCE TESTS

Blood Glucose, Serum Insulin, Growth Hormone and Free Fatty Acids

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Abstract. Blood glucose, serum insulin, growth hormone and free fatty acids (FFA) have been measured in eight young healthy subjects after oral glucose loads of 25, 50, 100 and 200 g. While the blood glucose areas only quadrupled, the insulin areas rose by factor of 16 through the eightfold increase in oral glucose load. In the early part of the tests blood glucose was the same after high and low doses, while serum insulin was much higher after large doses. The significance of the I/G ratio in oral glucose tolerance tests is discussed in the light of the results. The serum growth hormone suppression phase increased with increasing glucose loads in male as well as in female subjects. In female subjects, however, this regular pattern was obscured by intermittent peaks. The serum FFA suppression phase also increased as glucose loads increased. Serum FFA patterns were identical in male and female subjects.

The oral glucose tolerance test has been the most employed tool for the critical evaluation of carbohydrate tolerance during more than 50 years. Hansen (7) showed in 1923 in her excellent monograph that the rise of the blood sugar varied within very narrow limits when the dose exceeded a certain size. Since then few and somewhat conflicting reports have appeared on the role of different quantities of the oral glucose load (1 3 6, 15 16, 17). The commonest dose used in Britain is 50 g, 70 g is often employed in Scandinavian countries, 100 g in USA. A weight-related glucose dose, e.g. 1 75 g/kg or per kg ideal b wt., is also used by many investigators. This disparity has often been felt as a hindrance to interpretation and comparison of results obtained in various studies. During the last ten years the situation has become even more complicated when the oral glucose tolerance test became the natural procedure in the evaluation of insulin and growth hormone responses in various physiological and pathological conditions. In 1963 Hales and Randle (6) and very recently two other groups of workers (1 3), have investigated the insulin response to varying glucose loads. These results are, however conflicting to some degree.

In the following a report will be given of the effect on blood glucose and serum insulin of varying amounts of glucose given in the oral glucose tolerance test. Serum growth hormone has been determined in order to elucidate the problem of primary depression and secondary rise after administration of various glucose loads. Serum free fatty acids (FFA) have also been estimated in these studies.

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SUBJECTS AND METHODS

Five male and three female healthy normal-weight subjects, aged between 19 and 32 years, were studied.

Blood glucose was determined by glucose oxidase method (4). Serum insulin and growth hormone were estimated by radioimmunoassay employing wick-chromatography (12). Human insulin (Novo) was used for the preparation of insulin standards and a Wilhelmi preparation (HS 968 C) for growth hormone standards. FFA in serum was determined by the spectrophotometric method of Laurell and Tibbling (9).

Procedures

All glucose tolerance tests were performed in the fasting state during the six hours between 8 a.m. and 2 p.m.

Table I. *Average blood glucose concentrations in the eight subjects*

The 15-minute sample was obtained only in four subjects this value has not been used in the calculation of the area of increase

	0′	15	30′	60	90	120	150′	180′	210	240′	270′	300	330′	360′	Area	%
25 g																
Mean	76	130	141	109	86	73	72	72	77	78	80	80	81	81	108	100
S.E.M.	2.4	14.5	13.8	9 0	6 0	2.9	2.0	1.5	1.2	1.6	1 7	2.1	2.5	2.3		
50 g																
Mean	78	131	152	135	109	90	78	71	71	75	79	79	81	81	174	161
S.E.M	2 0	5.9	8.0	12.4	10.5	7.9	5.2	3.0	3.0	2.4	1.8	1.6	1.3	1.9		
100 g																
Mean	77	129	168	162	132	110	86	77	74	71	75	77	79	79	265	245
S.E.M.	1.6	5.3	11.0	13.2	11.3	10.9	6.8	4.6	5.9	4 1	2.2	2.0	2.2	2.1		
200 g																
Mean	78	141	156	161	138	131	128	124	107	89	86	72	75	77	418	387
S.E.M	1.6	1 7	13 6	18.0	13.2	10 3	8.4	6 5	7.5	10.8	8.8	6.4	8.6	6 0		

The subjects were not allowed to rise from their beds during the test or the preceding 12-hour fasting period.

Venous blood was collected from indwelling catheters at ½-hour intervals during six hours and immediately chilled in iced water After clotting and separation at +4°C, serum was stored at −20°C. In addition, whole blood samples were frozen in tubes with NaF for blood glucose determinations.

In two subjects blood samples were collected at 5-minute intervals for closer scrutiny of interrelationships between the investigated parameters.

25 50, 100 and 200 g glucose were given to each subject on different days. In four of the subjects the different glucose loads were given in increasing and in the other four in decreasing quantities. The glucose was always dissolved in 400 ml water.

RESULTS

Blood glucose values

The mean values from the total series, males and females, appear in Table I and Fig. 1 The 15- as well as the 30-minute values from the 4 tests were very similar The return to fasting values was somewhat delayed with increasing doses. This also applied to the return to the 120 mg% level. The areas of increase over fasting levels rose approximately *fourfold* through the *eightfold* increase in glucose loads (Fig. 2)

Serum insulin values

The corresponding serum insulin values from the 4 tests are seen in Table II and Fig. 1 In contrast to the glucose concentrations, insulin values were clearly separated at the 30-minute point. It can be seen that the areas of increase over fasting levels of insulin rose *sixteenfold* in response to the *eightfold* increase in glucose load. The relative areas of increase of glucose and insulin appear in Fig. 2.

Table II. *Average serum insulin concentration in the eight subjects*

The 15-minute sample was obtained only in four subjects; this value has not been used in the calculation of the area of increase

	0′	15	30	60	90	120	150	180	210	240′	270′	300′	330′	360	Area	%
25 g																
Mean	12	81	61	37	16	12	8	8	12	10	8	9	10	11	78	100
S.E.M.	2.0	16.1	12.1	8.4	2.5	2.1	1.6	2.1	4 1	2.8	1.9	2.5	1.5	3.2		
50 g																
Mean	11	93	91	63	49	31	17	10	8	9	11	9	9	9	196	251
S.E.M	2.1	6.8	19 5	7 1	9.2	10.4	5.3	2.5	1.2	1.8	2.5	2.0	2.0	2.3		
100 g																
Mean	13	98	120	175	128	89	44	25	18	10	8	9	7	9	508	651
S.E.M.	3.1	20.1	17 7	54 1	32.9	20.9	10.5	5.2	4 0	2 8	2 0	2.3	1.5	2.4		
200 g																
Mean	9	115	155	192	168	186	197	165	110	66	45	25	20	18	1 237	1 586
S.E.M.	2.1	12.5	32.1	43 9	44.1	55.3	52.9	39.0	23.5	14 1	12.0	5.5	6.4	4.8		

Table III *Individual I/G ratios calculated from the areas of increase after the four different glucose loads*

Case no.	25 g	50 g	100 g	200 g
1	1.40	1.32	1.60	4.31
2	0.50	0.61	1.39	3.17
3	0.38	1.40	3.75	3.78
4	0.35	1.54	1.10	1.37
5	0.22	0.68	1.01	1.42
6	0.83	0.85	1.92	2.14
7	1.19	1.47	2.73	3.41
8	1.23	0.82	1.32	2.28
Mean	0.79	1.09	1.85	2.74

Insulin/glucose (I/G) ratios

As could be expected from Fig. 2 and seen in more detail in Table III, the mean insulin/glucose (I/G) area increased from 0.79 to 2.74 with increasing glucose loads. The individual I/G ratios increased by factors ranging from 2 to 10.

Serum growth hormone values

The results from the male subjects are seen in Fig. 3. The postprandial rise in serum growth hormone is delayed with increasing glucose loads. It was evident from the individual values that fasting blood glucose concentrations had always been reached before growth hormone rose. This conclusion was confirmed in the experiments on two of the subjects (in 8 glucose tolerance tests) from whom 5-minute interval samples were collected.

There was no relationship between the attained growth hormone maximum and the administrated quantity of glucose.

While the growth hormone increase pattern was very consistent in the male subjects, another picture was obtained in the female subjects. Although the same general pattern of increasing delay could be made out, it was obscured by intermittent glucose-unrelated peaks (Fig. 4).

Serum FFA values

In contrast to the differing results obtained in growth hormone patterns between female and male subjects, identical FFA patterns were found, i.e. after the initial suppression phase the rise in FFA occurred always, both in male and female subjects, after the return to fasting levels of glucose and insulin (Fig. 5).

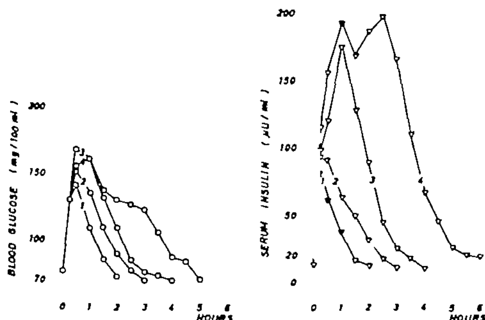


Fig. 1 Average blood glucose and serum insulin curves from the 25 (*1*), 50 (*2*), 100 (*3*) and 200 g (*4*) oral glucose tolerance tests.

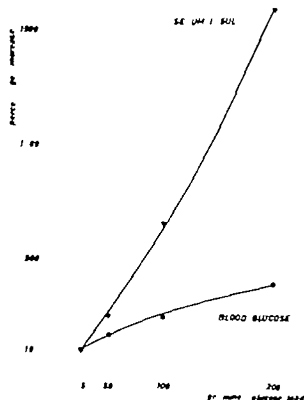


Fig 2 Average percentage changes in areas of increase of serum insulin and blood glucose through the different glucose loads.

Results f om short-interval sampling

Two male subjects were studied with 5-minute interval sampling. The time it took for serum insulin to return to fasting values was on the average 28 min longer than for blood glucose. In the 8 glucose tolerance tests made on these two subjects insulin had reached the pre-load level at the same time as the blood glucose in 4 and somewhat later in the 4 other tolerance tests. The average growth hormone and FFA rises occurred 41 and 59 min, respectively after the return of blood glucose the rises occurred in all instances after fasting blood glucose concentration had been reached.

DISCUSSION

It is apparent from our results that the attained *maximum* blood glucose concentrations, which occur at about 30 min after glucose administration, are not affected by the large increases in glucose loads from 25 to 200 g This is in accordance with Hansen's results (7) as well as those of Castro et al. (1) who compared 50, 75, 100 g as well as 1 75 g/kg. Similar results were obtained by Chandalia and Bosbell (3) after 30 and 45 g glucose/m² BSA.

In contradistinction, the early serum insulin values obtained in the present study had separated widely already at 30 min. This is in accordance with the findings of Hales and Randle (6), who compared the effect of 50 and 100 g oral glucose.

The areas below the glucose and insulin curves also changed very differently through the increase in glucose loads. While the blood glucose areas quadrupled, the serum insulin areas increased by a factor of 16. Castro et al. (1) found also increasing serum insulin areas with increasing glucose loads. They stated, however that no

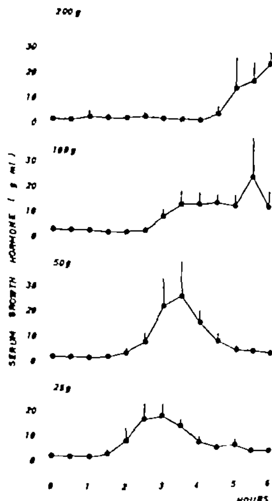


Fig 3 Average serum growth hormone concentrations in five male subjects. The bars signify 1 S.E.M.

further increase will occur when the glucose load exceeds 100 g. This assumption is based on relatively small increments in loads above 100 g. The enormous difference in insulin areas found in our series of 100 and 200 g glucose tolerance tests seems to disprove their statement.

The disparity between identical early blood glucose values and highly increasing serum insulin concentrations after increasing oral glucose loads is noteworthy. It demonstrates that other factors than the blood glucose govern the insulin response to *oral* glucose, for instance intestinal factors (10). The importance of such factors was originally suggested by the finding that i.v. glucose infusions induced very much smaller insulin responses than oral tests with comparable hyperglycemia. We believe therefore that the use

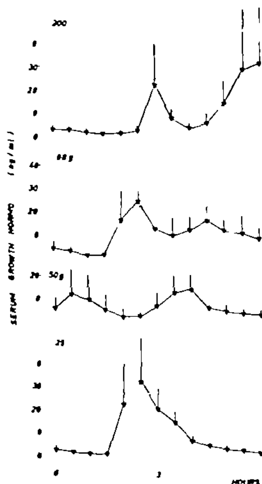


Fig. 4. Average serum growth hormone concentrations in the three female subjects. The bars signify 1 S.E.M.

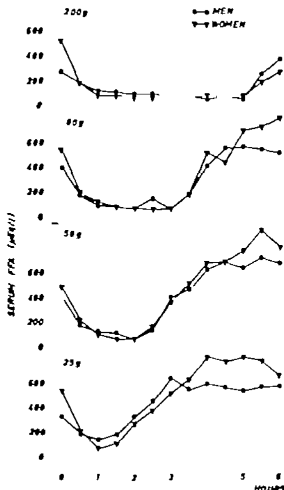


Fig. 5. Average serum FFA patterns in the five male and three female subjects.

of I/G ratios in oral glucose tolerance tests is questionable. When non-diabetics and diabetics of the same weight are compared, using identical oral loads, the difference in pancreatic stimulation will be due solely to the higher glycemia of the diabetics. However, the glycemic stimulus per se is small and obviously the I/G ratio in diabetics is underestimated. The fact that the calculated I/G ratios from our series increase steeply with the oral glucose load and with very varying individual factors ranging from 2 to 10, shows in itself in our opinion that oral I/G ratios must always be used with the greatest caution.

The results of the growth hormone determinations from the five male subjects confirmed the finding of Hunter et al. (8) that the postprandial

rise of serum growth hormone occurs with increasing delay as the oral glucose load is increased. In contrast to Hunter et al. we found no indication that the growth hormone maxima obtained also increased with the glucose load.

In the 20 glucose tolerance tests made in the five male subjects, serum growth hormone never rose before glucose had returned to approximate fasting levels. This regular pattern was not obtained in the female subjects, who exhibited sudden peaks with no relation to changes in the blood glucose. The erratic growth hormone pattern in females has earlier been demonstrated in other situations: during rest (5) during exercise (2) and during infusion of arginine (11)

Hunter et al. (8) suggested that the rise in FFA after oral glucose was dependent on growth hormone. However our results are in agreement with investigations made in other experimental conditions (13 14) showing that this is not the case.

While the serum growth hormone pattern differed in male and female subjects, identical patterns were obtained for the FFA suppression and secondary rise. This indicates that the inhibitory effect of insulin on lipolysis is of greater importance than the changes in serum growth hormone for the postprandial FFA pattern.

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BUDD CHIARI SYNDROME AND POLYNEURITIS

In Vivo Diagnosis of Hepatic Vein Stenosis

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Abstract. A 39-year-old man with an obstruction of the hepatic vein with concomitant ascites and oedema of the lower extremities is described. The cause seems to be congenital stenosis of the hepatic vein. At surgery there were no possibilities of relief. One cause of that failure may have been bleeding from collateral vessels caused by an earlier laparotomy. The literature on the Budd Chiari syndrome is briefly reviewed. Modern surgery combined with angiographic and catheterization techniques makes this grave disorder potentially curable one. This should especially be possible in the case of the above mentioned congenital strictures and webs, which in increasing numbers are diagnosed. The other and various causes of this syndrome, including malignancies, septic thrombosis etc., will most likely be diagnosed at autopsy. No connection between polyneuritis and this syndrome has been mentioned in the literature, as far as we can find. The origin of the polyneuritis in our case remains undeter mined. One may speculate whether disturbed hepatic metabolism may be the cause. The co-existence of two separate diseases must also be considered.

Budd (1) was the first to describe this syndrome. In 1857 he reported three cases and in 1899 Chiari described it more completely reporting three of his own cases and collecting seven others. The syndrome is characterized by stenosis of the hepatic veins, usually accompanied by ascites.

CASE REPORT

A 39-year-old man, born 1930, working as truck driver. Previous medical history of no interest until 1965. At that time moderate hypertension was recorded. He was also overweight—125 kg (height 182 cm). Examination for suspect Cushing syndrome at another hospital was carried out without any positive findings.

In Sept. 1969 the patient began to suffer from various sensations from the toes and the fingers, "like pins and needles". Gradually he became unable to move his toes, he was also handicapped by not being able to feel the ground beneath him. From April 1970 he had to use sticks when walking and could not climb stairs alone.

In Sept. 1970 he was admitted to the University Hospital, Uppsala. In his medical history w did not find either abuse of alcohol or any exposition to substances associated with suspicion of causing polyneuritis.

CLINICAL FINDINGS

Heavy oedema was found in the lower legs and feet, BP was 135/95 mmHg. There were telangiectases on both cheeks. The abdomen was enlarged due to ascites. The strength of the flexors and the extensors of the hips, knees and ankle joints was bilaterally diminished and the sensitivity of the legs, especially the vibration sense, as impaired up to the level of crista iliaca on both sides. Babinski' sign was negative on both sides No signs of mental confusion or instability were found.

LABORATORY INVESTIGATIONS

Hb concentration and leucocytes were normal. ESR (Westergren) changed from 10 to 45 mm. Bleeding and coagulation times were normal. The thrombocytes were approximately 500 000/mm³ Urine examinations ere negative for glucose and proteins (incl. Bence Jones protein). Urine sediment was normal. Serum electrolytes as well as serum creatinine ere normal. Serum bilirubin was quite normal, approximately 0.6 mg% (he as never icteric), but the alkaline phosphatases were slightly raised to 5.9–6.4 Bodansky-Lagen units. Australia antigen test as well as the test for antinuclear factors ere negative. The bromsulphthalein load test showed retention of 46% after 120 min, seriously impaired function. Total serum albumin was 3.1 g% total protein content of the serum 4.5 g%. The serum electrophoresis contained broad based, raised γ-globulin fraction. I glucose tolerance test was normal.

T biopsies f peripheral nerves ere taken. They showed, however nothing of interest. A scintigram of the liver showed defect in the uptake in the upper central part of the liver. Cytology performed on the ascites fluid



Fig 1. Cineradiogram showing the obstruction of the hepatic vein, indicated by the arrows. *RA*, right atrium; *IVC*, inferior cava vein, ↔ indicates the collateral vein.

of the abdomen showed large numbers of lymphocytes, granulocytes and mesothelial cells but no malignant cells. Radiology: splenoportography showed a pressure in the spleen of 49–50 cm H_2O. The intra-hepatic branches of he vena porta were dislocated within central parts of liver raising suspicion of an expansive process in the Abdominal aortography showed normal renal, mesenterial and coeliacal vessels.

An explorative laparotomy was then performed, in hich biopsies of the liver parenchyma as ell as from the pancreas were taken. N signs f malignancy were found—the cells of the liver rather supported the view of venous stasis and slight unspecific inflammation.

At this point strong suspicion arose of obstruction in hepatic veins or and in the inferior caval vein. There was no clinical evidence of constrictive pericarditis. Catheterization and angiography of the right atrium and retrograde examination of the inferior caval vein and the vena hepatica revealed the following findings. At the junction of the vena hepatica with the vena cava inferior there was a compression of the cava to one-third of its normal idth. The hepatic veins were found to be united to one common branch—and this branch was stenosed. Some collaterals were also found to this vein, entering the cava some 10 cm below (Fig. 1). The mean pressure recorded in the right atrium as 2–4 mmHg, in the inferior cava vena 6 mmHg, and in the hepatic vein 22 mmHg. A pressure gradient of 19–20 mmHg as measured between the right atrium and the hepatic vein distal to the stenosis was thus verified. At this point it could reasonably be said that Budd Chiari syndrome existed.

The patient received spironolactone and furosemide and the oedema of the lower extremities gradually disappeared. The ascites of the abdomen, however, only partly responded to diuretic treatment. He was also trained in physical exercise due to the above mentioned instability of the legs. His weight dropped from 109 kg in Nov 1970 to 80 kg in mid-March 1971.

A new operation was performed in May 1971 at the Department of Thoracic Surgery. The above mentioned stenosis of the inferior vena cava was found to be caused by an impression of the liver. The radiologically established stenosis of the hepatic vein was localized few cm below the upper surface of the liver. Although clamps were placed both on arteria hepatica and the vena porta, bleeding was so extreme that a reconstruction of the stenosed vessel was impossible. Biopsies taken from the area contained normal liver cells. The patient recovered well and was discharged for convalescence. Ambulatory control at the Department of Internal Medicine in Sept. 1971 showed unchanged status, with moderate ascites but no peripheral oedema.

DISCUSSION

Many and various causes of the Budd Chiari syndrome have been described. Feingold et al. (3) report a case in whom a right atrial tumour probably primarily a cardiac neoplasm extended downwards in the vena cava inferior. The patient underwent open heart surgery but died post operatively due to thrombocytopenia and grave abnormalities in the clotting of the blood.

The authors conclude that with available angiographic techniques it becomes imperative to search out definitive causes for the syndrome—since it may be possible to cure an otherwise lethal disease.

Young (11) describes two cases of young patients, aged $4\,^1/_2$ and $9\,^1/_2$ years, respectively suffering from acute lymphocytic leukaemia. They were both under *treatment* with vincristine sulphate methotrexate mercaptopurine and prednisone. The youngest patient initially had positive cultures for Candida albicans in her throat, urine and stools; at autopsy thrombosis of the hepatic veins due to masses of Aspergillus appeared. The older patient similarly had septic conditions—Mycoplasma–Pseudomonas, and at autopsy widespread abscesses in the liver brain lungs and thyroid etc., were found. The author mentions that, as in our case, liver function tests showed mild to moderate impairment. Jaundice was never striking—the highest value for serum bilirubin was 2.6 mg %. As Parker (9) pointed out, jaundice was present in 28 % of 164 patients with the Budd Chiari syndrome—and seldom more than

slight. Besides the Aspergillus septicemia with septic thrombosis, chronic leukaemia per se is reported as a cause of the Budd Chiari syndrome by Palmer (8) Intravascular leucostasis predisposes to vascular thrombosis and infarction.

Volpe et al (10) report a case of a 20-year-old man with a low inferior vena cava thrombosis and a Budd Chiari syndrome in connection with visceral thrombophlebitis migrans. Severe portal hypertension prompted surgical intervention—in this case a spleno-renal shunt was performed. The patient died postoperatively due to oesophageal bleeding. Section revealed an obstructing web with a 5 mm opening just cephalad to the entrance of the hepatic veins into the inferior vena cava.

There have been at least 60 reported cases of patients with obstructing webs of this kind—many presumably of congenital origin.

Johansson (4) describes a case in whom an obstruction at the level of vertebra Th 10 at the inferior vena cava existed. Caudal to that obstruction dilated hepatic veins branched off and united with one stenosed vessel cranial to the obstruction entering the vena cava. At operation, and later at autopsy it was found that cells of the connective tissues—resembling embryonal tissue—caused the obstructions and stenosis.

Kimura (5) discusses the development of the hepatic veins and the inferior vena cava in the foetus and finds it reasonable that in these complicated vascular changes developmental defects are likely to occur

Mühe et al. (7) on these assumptions, classify the congenital obstructions of the inferior vena cava as follows: a) *incomplete stricturing membrane* of the vena cava inferior b) complete but thin membrane and c) a complete and thick membrane of the cava. In all cases the left and medial hepatic veins were completely stenosed.

However one must not forget that in many cases, as discussed by Leopold et al. (6) malignant tumours may develop metastases or primary tumours may grow from the kidneys, lungs etc. and cause a Budd Chiari syndrome The possibility of a subphrenic abscess is also considered by these authors as a cause of the Budd Chiari syndrome.

Hoyumpa et al. (2) have recently added two cases of patients on oral contraceptives to six previously recorded. Five of the eight patients had been on preparations containing more than 0.05 mg oestrogen and two on low-oestrogen-containing products, in one the proportion of oestrogen and progesteron was not stated.

The duration of exposure to an oral contraceptive ranged from two weeks to eight years. They conclude that although direct evidence is lacking the possibility of a causal relationship between hepatic vein occlusion and the use of oral contraceptives is worthy of consideration Of the eight cases associated with oral contraceptives two have pparently recovered.

The pathology of the liver appears in the Budd Chiari syndrome according to Hall (1) as follows. The liver is smooth and congested and extremely firm. On the cut surface areas of congestion alternate with irregular yellow patches of fatty degeneration. Microscopic examination reveals in the usual case marked congestion with atrophy and necrosis of the central part of the hepatic lobules. The disease (in its idiopathic form) occurs with equal frequency in the two sexes, usually between the age of twenty to forty years. In the *acute* form the onset is sudden with abdominal pains, nausea, shock, vomiting, gradually followed by ascites, tenderness and enlargement of the liver and spleen—delirium coma and death ensue in one to four weeks In the *chronic* form the onset is more gradual with epigastric pains, ascites, tender enlargement of the liver and envelopment of the collateral circulation. Jaundice is rarely observed, coma, delirium and death usually follow within about six months.

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REHYDRATION AS A DIAGNOSTIC AND THERAPEUTIC MEASURE IN HYPERCALCEMIA

Including an Assessment of the Calcium-lowering Effect of Porcine Calcitonin

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Abstract. Two patients in hypercalcemic crisis received fluid replacement therapy which sufficed to control their condition. Both of them developed marked polyuria even before they were clinically rehydrated and large amounts of calcium were demonstrated in the urine. A third patient, who clinically presented with the most pronounced symptoms of hypercalcemia, did not produce polyuria or hypercalciuria when rehydrated. It is suggested that the presence or absence of polyuria upon rehydration may serve to distinguish between hypercalcemic crisis sensu stricto and hypercalcemia with less rapid calcium mobilization. When the two first mentioned patients had been brought into steady state with stable, but elevated serum calcium and constant, but abnormally high daily excretion of calcium in the urine, porcine calcitonin was administered. One patient responded with drop in serum calcium and phosphaturia, both parameters reverting to pretreatment values upon cessation of the drug. In the other patient serum calcium was abruptly normalized, urinary excretion diminished and the effect was sustained.

In hypercalcemia due to increased osseous resorption the mobilization of calcium from bone proceeds at a different and possibly varying rate in each patient. The severity of the clinical symptoms and the level of serum calcium seem to be poorly correlated to the total amount of excess free calcium in the body at a given time and to the rate of bone destruction. Nevertheless, that is more often than not the only available information on which treatment has to be instituted, as it is impossible to foretell the development of the condition and whether—and how fast—the level of serum calcium is going to rise. It is commonly stated that a serum calcium of 17 mg/100 ml or more is life-threatening; however the rate of change of serum calcium is probably of equal importance.

The fact that the amount of calcium to be neutralized is unknown may account for some of the therapeutic failures. Another problem is that the calcium-lowering effect of the various agents used is ill defined. In hypercalcemia the level of serum calcium may ary due to change in calcium mobilization or because of a compensatory mechanism initiated by the organism irrespective of the specific treatment given. A reduction of serum calcium in connection with administration of a certain amount of a calcium-lowering agent cannot be interpreted unless it has been established that a steady state has been achieved between in- and outflux of calcium in serum.

It is hypothesized that the polyuria and hypercalciuria accompanying hypercalcemia serve as compensatory mechanisms enabling the organism to excrete excess free calcium with complete efficacy as long as normohydration is maintained, and that the amount of calcium recovered in the urine corresponds to the rate of bone destruction.

If this theory is alid, two parameters are available for evaluating the severity of hypercalcemia and the immediate treatment should aim at restoring and maintaining fluid balance.

Satisfactory results of treatment of acute hypercalcemia have been obtained by Suki et al. (8) who used large doses of furosemide in association with fluid replacement. This treatment, however needs very close supervision in iew of the risk of electrolyte derangements.

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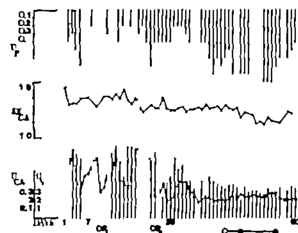


Fig. 1 Case 1. Variations of serum calcium, diuresis and urinary excretion of calcium and the response to calcitonin.

U_P, Urinary phosphorus (g/l) (bars); SE_{CA}, serum calcium (mg/100 ml)· U_{CA}, urinary calcium (g/l) (bars)· U_V, volume of urine (l) (bottom curve)· *OP* subtotal strumectomy· *OP* exploratory thoracotomy· ○ calcitonin in a gelatin diluent 16 MRC i.m. t 8 a.m.; ● calcitonin in gelatin diluent 16 MRC i.m. t 8 a.m. and 8 p.m.

From day 1 to 55 sodium-potassium diphosphate was given by mouth daily in amounts varying from 9.9 to 3.3 g. From day 9 to 39 prednisolone was given 30 mg/day and from day 40 to 47 25 mg daily

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Calcitonin is a hormone extracted from the thyroid gland (1) and shown to act—at least in some respects—as an antagonist to the parathyroid hormone in experiments undertaken with rats (4) A lowering effect of calcitonin on serum calcium in human beings has also been demonstrated (5)

To illustrate the concepts outlined above and to demonstrate the results obtained with calcitonin, three cases are presented here.

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A 46-year-old woman was hospitalized in June 1970. In 1965 diagnosis of thyrotoxicosis had been made elsewhere; no efficacious treatment had been possible due to lack of co-operation on the part of the patient. She had sporadically taken methimazole or propylthiouracil. Two days prior to admission she had suddenly been taken ill with fever, nausea, vomiting, backpain and frequent voiding. A suspicion of acute pyelonephritis was not supported by the laboratory findings. At clinical examination she was found to be lethargic, semistuporous, moderately dehydrated and obviously thyrotoxic with a nodular goiter. Pertinent laboratory findings included: PBI 1..t µg/100 ml (control 3 days later 20.6 µg/100 ml), T resin uptake 143% (normal upper limit 121%), 4-hour uptake of I-132 in the thyroid 54% (normal upper limit 45%), serum cholesterol subnormal, creatine in urine 1 1 g/24 h (normal upper limit 0.150 g/24 h), serum magnesium 1.3 mEq/l. Serum phosphorus was in the lower range of normal, alkaline phosphatases not elevated, serum calcium 16.1 mg/100 ml. Appropriate investigations revealed no signs of malignant disease, skeletal destruction, stones in the kidneys or sarcoidosis. Thyrotoxicosis with an associated adenoma of the parathyroid was considered to be the most likely diagnosis.

On day 9 a subtotal strumectomy was done, but no parathyroid adenoma was found. In connection ith the operation iodine, serpasil and steroids were given; the steroids continued for five eeks. No antithyroid medication had been given prior to operation. An exploratory thoracotomy two weeks later revealed no parathyroid adenoma. About this time the patient appeared clinically to have become euthyroid. Hypercalcemia, although much less severe, was, however still present and has persisted up to the last control 9 months later The clinical recovery has been complete. Tentative diagnoses are thyrocalcitonin deficiency or parathyroid adenoma.

The hypercalcemia was treated with fluid replacement i. or by mouth. I. fluids consisted of 0.9% saline and 5% dextrose in water. T prevent hypokalemia a small amount of potassium was given by mouth. Normohydration was restored and the fluid balance maintained simply by replacing measured and calculated losses. Lethargy and nausea disappeared promptly and the patient' condition remained satisfactory The polyuria was impressive: it amounted to as much as 9 l/24 h. Despite the fact that urinary excretion of calcium varied considerably while the patient was still thyrotoxic, and occasionally reached high values (max. 1.8 g/24 h) serum calcium was kept comparatively stable. It never reached the initial value again (Fig. 1). No electrolyte derangements ere incurred.

A diphosphate solution was given by mouth, probably without effect. The patient has, later during control period, received diphosphate orally as sole treatment

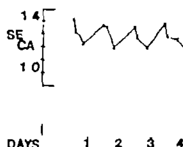


Fig 2 Case 1 Variations of serum calcium during four days treatment with porcine calcitonin in gelatine diluent, 16 MRC i.m. daily at 8 a.m. Serum calcium measured t 8 a.m 10 a.m. and 4 p.m., on day 4 also at midnight.

SE_{CA}, Serum calcium (mg/100 ml).

without any reduction of serum or urinary calcium. Equally she has proved refractory to suppression test with 40 mg prednisolone for 10 days.

Six weeks after hospitalization, when serum and urinary calcium had become stabilized at an elevated level of approximately 14 mg/100 ml and 0.45 g/24 h, respectively treatment with porcine calcitonin (Armour) 16 MRC (Medical Research Council) at 8 a.m. was begun and continued for four days. The effect on serum calcium was maximal after 8 hours and wore off within 4 hours. On four consecutive days serum calcium dropped from approximately 14 mg/100 ml at 8 a.m. to approximately 12 mg/100 ml at 4 p.m. (Fig. 2), save on the fourth day of treatment when the minimum value was not reached till midnight. The calcium-lowering effect is not reflected in Fig. 1, here the morning values are plotted, till an additional amount of 16 MRC at 8 p.m. was given; it was continued for 11 days. Fig. 1 shows the level of serum calcium 12 hours after administration of calcitonin. A phosphaturic action of the drug was demonstrated. As regards urinary calcium the values were slightly lower than those recorded before and after treatment.

The patient was kept on a diet containing maximally 150 mg calcium/day.

Case 2

A 59-year-old man was hospitalized in Oct. 1970 with complaints of fatigue, aches and pains in the muscles and joints for three months. Both knees and the right ankle were swollen. Otherwise physical examination revealed nothing of interest. Pertinent investigations included ESR 125, immune electrophoresis of serum M-type protein between the α-2- and β-fraction (same fraction demonstrated in the urine), total serum protein 7.3 g/100 ml. Two of the ordinary agglutination tests for the rheumatoid factor in plasma were positive. Serum creatinine 0.9 mg/100 ml, serum calcium 9.4 mg/100 ml. Bone marrow hyperplastic with an increased number of plasma cells. X-ray of the skeletal system showed no destruction. I. v. pyelography normal. A tentative diagnosis of multiple myeloma was made, but rheumatoid arthritis could not be excluded. The patient was discharged without treatment.

In Jan. 1971 he was readmitted with a history of polyuria of one month duration. He appeared ill, dehydrated, slow cerebrated with incoherent speech, hyperactive deep tendon reflexes and plantar reflexes of the extensor type. Alkaline phosphatases were normal, serum calcium 17.8 mg/100 ml (control 18.4 mg/100 ml). Treatment with cyclophosphamide 300 mg/d. and Prednisolone 60 mg/d. was started, the latter discontinued after one week. Two months later he was discharged in good condition on a maintenance dose of cyclophosphamide. At weekly controls in the Out-patient Department he has had no complaints save for recurring arthritic symptoms in the knee joints. The M-type protein fraction in serum has been reduced. Total serum protein is unchanged.

Hypercalcemia was controlled with fluid replacement as outlined in case 1. Maximal urinary excretion of calcium was noted during the first 24 hours when it reached 2.6 g (Fig. 3) and decreased thereafter slowly. Serum calcium fell steadily. Constant levels of serum and urinary calcium were attained after a couple of weeks (Fig. 3).

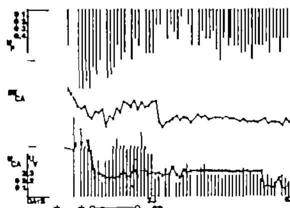


Fig. 3. Case 2. Correlation between serum calcium, urinary calcium and volume of urine and the response to calcitonin.

★ Prednisolone (60 mg/d); ○ calcitonin in gelatin diluent 13 MRC i.m. at 8 a.m. and 8 p.m. on days 11 and 12, followed by 26 MRC twice daily till day 24. ● calcitonin 160 MRC 8-hourly (on day 64 130 MRC 8-hourly). Other symbols as in Fig. 1.

Cyclophosphamide, 300 mg/d. instituted on day 1 was discontinued on day 19. From day 25 onwards a maintenance dose of 100 mg/d. has been given.

Serum creatinine was initially 4.2 mg/100 ml and fell gradually in three weeks to 1.1 mg/100 ml. On day 12 creatinine clearance was 59%, on day 22 it had increased to 87%. BUN fell from 106 to 39 in two weeks. No electrolyte derangements were incurred, but inadvertently the patient had become somewhat overhydrated during the first week. Overhydration was corrected on days 9–10 and 11 simply by reducing fluids, after which he lost approximately 3 kg in weight.

On day 12 calcitonin treatment in a comparatively small dose was started. Stable levels of serum and urinary calcium had not been achieved at that point, so the immediate effect, if any, cannot be interpreted. However, a steady state had been reached before calcitonin was discontinued two weeks later (Fig. 3) and no variations were noted in these two parameters upon cessation of the drug. A new attempt with calcitonin was made on days 29 and 30 when the patient received 160 MRC three times daily. In connection therewith serum calcium was abruptly normalized, it fell from 13.3 mg/100 ml to 10.4 mg/100 ml, and urinary excretion diminished to about half of the pretreatment values (Fig. 3). Serum magnesium and serum phosphorus, which had hitherto constantly been subnormal, promptly rose to normal values. No phosphaturia was noted. Quite unexpectedly these parameters remained normal, save for urinary calcium which was still in the upper range or slightly above. Urinary excretion of calcium remained borderline and it was decided to try to establish the causal relationship between the administration of calcitonin and the apparent normalization of calcium balance. Consequently on day 64 calcitonin 130 MRC 8-hourly was given, resulting in a drop of

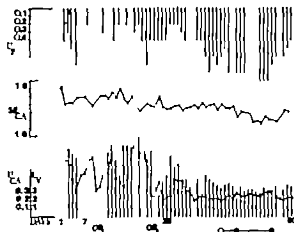


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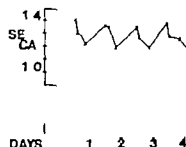


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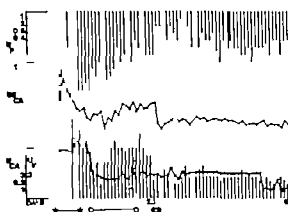


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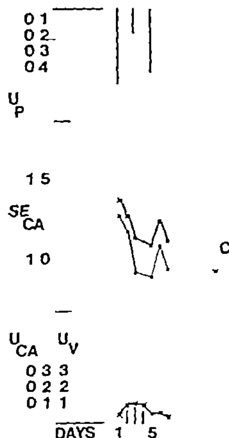


Fig. 4. Case 3. Lack of correlation between the level of calcium, on the one hand, and the amount of urinary calcium and volume of urine, on the other. C Serum calcium corrected for protein deficit. Other symbols as in Fig. 1.

urinary excretion of calcium to 0.06 g/24 h for one day only. The other parameters remained unaffected. The patient has been kept on a diet containing maximally 150 mg calcium/day.

Case 3

A 65-year-old woman was hospitalized in Feb. 1971. Two years previously palliative irradiation of the vertebral column had been administered because of osteolytic metastases from an inoperable cancer of the breast diagnosed shortly before. On treatment with prednisolone 15 mg daily and weekly injections of anabolic steroids she had done tolerably well. Three weeks before admission her condition had begun to deteriorate with episodic mental confusion, progressive drowsiness and fever. Frequent voiding had been noted. On clinical examination she was comatose, dehydrated and continuously regurgitating. Serum calcium was 1...5 mg/100 ml, control 13 mg/100 ml (corrected for protein deficit 14 mg/100 ml). Total serum protein was initially 6.3 g/100 ml, after rehydration 3.9 g/100 ml. Prednisolone 15 mg/d. was continued parenterally, later by mouth. After four days she had cleared up mentally but remained weak. She was transferred to a nursing department where she died after eight weeks. At autopsy a meningeal carcinomatosis was demonstrated.

The hypercalcemia was treated with fluid replacement as in cases 1 and 2. Diuresis remained modest. Urinary excretion of calcium did not exceed normal limits. The normocalcemia established (Fig. 4) was maintained by adequate hydration.

Serum creatinine, initially 3.0 mg/100 ml, fell slowly to 1.1 mg/100 ml in two weeks.

As in the other two cases a diet low in calcium was given.

DISCUSSION

In all three patients symptoms were severe enough to warrant prompt treatment of the hypercalcemia per se, a treatment which it would have been difficult to quantitate.

Judging by urinary excretion, calcium mobilization fluctuated considerably in case 1 during the first month, indicating that it would have been impossible to establish a fixed dosage schedule of any specific calcium-lowering agent.

On the other hand, in case 2 the rate of bone destruction was probably constant. The large quantities of calcium excreted initially may partly be derived from extraskeletal deposits. The low excretion around day 14 may have been caused by the overhydration which literally washed out these deposits. The constant excretion later is considered representative of the actual rate of bone destruction. Whether or not the prednisolone administered during the first eight days contributed to the lowering of serum calcium is uncertain: serum calcium continued to fall nearly linearly for three and urinary calcium for six more days after cessation.

As regards case 3, calcium mobilization seems to have proceeded at a very slow rate: compared with the other two cases the urinary content of calcium was very modest. Serum calcium was, however, comparatively high and in discrepancy with the low urinary calcium. The severity of symptoms was partly accounted for by the underlying disease, but in the acute situation it may be difficult to evaluate the effect derived from contributory causes. Treatment of hypercalcemia of this type with EDTA, for instance, might readily have proved lethal.

Seen over a longer period, correlation between the urinary content of calcium and the magnitude of the diuresis is good in all three cases. It seems

justified to believe that in hypercalcemia the degree of polyuria indicates the severity of the condition.

Further it seems established that, if normohydration is maintained, the patient will be able to compensate for the increased bone destruction by increasing urinary excretion of calcium even during a prolonged period of massive calcium mobilization. It is suggested that treatment of hypercalcemic crisis should merely consist of fluid replacement.

It is further suggested that, if stable levels of serum and urinary calcium are maintained during several consecutive days, the latter may be regarded as a reflection of the actual rate of calcium mobilization which is then equally stable. Under these circumstances a quantitative evaluation of a calcium-lowering agent ought to be possible.

Porcine calcitonin has been tried and in case 1 the results are somewhat similar to those reported by other authors (2, 3). It is possible that the diphosphate solution given simultaneously may have enhanced the effect. Experiments with rats indicate the possibility hereof (7). The lowering of urinary excretion of calcium during the treatment period in patient 1 is so slight that no conclusions can be drawn. An enlargement of the calcitonin dose might perhaps have served to clarify whether or not it reduces calciuria.

At this point no explanation can be given for the result obtained in case 2, but a case with certain similarities has previously been reported by Pak et al. (6).

No adverse reactions to calcitonin were noted.

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Table I. *Clinical and laboratory findings in the subjects studied*

Pat. no	Age (y.)	Sex	PBI (µg/100 ml)	Cholesterol (mg/100 ml)	Resin uptake of T_3-^{131}I (%)	TSH (µIU/ml)	Thyroidal 24-h uptake of ^{131}I (%)	Treatment (mg/d.)	Duration of hypothyroid symtoms (y.)	(mo)
Control group										
1	42	♂[a]	5.4	231	37	<12.5	—	Thyranon 150		
2	47	♀	9.8	—	28	<12.5	—	Levaxin 0.30		
3	48	♀	9.2	341	32	<12.5	—	Levaxin 0.25		
4	59	♀	8.7	300	31	<12.5	—	Levaxin 0.15		
5	60	♀	7.0	283	26	<12.5	—	Levaxin 0.20		
6	62	♀	7.3	260	25	<12.5	—	Levaxin 0.15		
7	64	♀	8.2	349	28	<12.5	—	Levaxin 0.15		
8	65	♀	5.9	255	28	28.5	—	Thyranon 112.5		
9	67	♀[a]	9.8	211	33	17.3	—	Levaxin 0.20		
10	68	♀	12.5	247	29	24.5	—	Levaxin 0.20		
11	68	♀[a]	11.4	244	36	14.0	—	Levaxin 0.15		
12	68	♀	7.3	258	26	22.6	—	Thyranon 112.5		
13	75	♀	8.3	200	31	<12.5	—	Levaxin 0.15		
Hypothyroid group										
14	41	♂	3.6	224	33	118	27		0	6
15	44	♀	2.2	313	23	210	3.5		6	0
16	51	♂[a]	1.9	319	27	196	26		1	0
17	51	♀[a]	4.7	325	24	49	—		0	5
18	51	♀	3.6	560	24	77	5		0	1
19	56	♀	3.7	283	21	145	29		0	2.5
20	56	♀	4.8	308	29	43	8.4		0	4
21	57	♀	4.1	338	26	43	17		1	0
22	59	♀	3.2	355	26	189	16		0	4
23	59	♀	2.9	289	26	88	—		0	3
24	61	♂	<2.5	418	23	118	7		0	2
25	61	♀	3.8	351	20	70	13		0	4
26	63	♂	3.5	262	20	103	11		>1	0
	66	♂[a]	<2.0	637	23	172	4		>2	0
	71	♀	8.7[b]	262	17	90	—		1	0
	72	♀	1.9	489	24	175	3.5		0	4
30	74	♀	<2.5	343	24	68	1		0	2
31	76	♀	<2.5	436	19	—	8		0	2

[a] Hashimoto disease.
[b] High value probably due to iodine contamination.

Due to limitation in the amount of adipose tissue obtained by the biopsy technique, all experiments could not be performed on specimens from each subject.

Determination of PBI, cholesterol, resin uptake of ^{131}I labelled triiodothyronine and thyroid uptake of radioiodine at 24 hours were performed in non-fasting patients. The previously published values were used for the normal ranges (18). Human thyrotrophin (TSH) was measured by the double antibody technique described by Odell et al. (16). The normal range in our laboratory is 12.5–40 µIU/ml. Values lower than 12.5 µIU/ml could not be measured.

The following pharmacological agents were used in the incubations: l-noradrenaline bitartrate (Astra, S eden), phentolamine, Regitin (Ciba, Switzerland), N⁶ 2'O-dibutyryl cyclic 3',5' adenosine monophosphate (dibutyryl cyclic AMP) (Boehringer/Mannheim, West Germany), propranolol, Inderal® (ICI, England). Theophylline was obtained commercially

RESULTS

Effect of noradrenaline and α- and β-adrenergic antagonists

Basal glycerol release was of similar magnitude in the control and the hypothyroid groups (1.059 ± 0.189 and 0.980 ± 0.122 µmoles/g ww/2 h) (Table II) In contrast, lipolysis induced by noradrenaline (2×10^{-6} M) differed markedly between the two groups. In tissue specimens from the controls a significant stimulation (0.631 ± 0.200 µmoles/g ww/2 h, $p < 0.02$) was observed, while net glycerol was slightly reduced (-0.166 ± 0.087 µmoles/g ww/2 h, $p < 0.1$) in the hypothyroid group.

The addition of the β-adrenergic blocking agent, propranolol, at a concentration of 1 µg/ml re-

Table II. *Effect of noradrenaline and propranolol or phentolamine on the release of glycerol from subcutaneous adipose tissue from controls and hypothyroid subjects*

	Controls					Hypothyroid subjects				
Addition to medium	N	Mean ±S.E.M.	N	Mean diff. ±S.E.M.[b]	p	N	Mean ±S.E.M.	N	Mean diff. ±S.E.M.	p
None	12	1.059±0.109		—	—	16	0.980±0.122		—	—
l-noradrenaline (2 10^{-6} M)	12	1.728±0.284	11	0.631±0.200	<0.02	18	0.761±0.129	16	−0.166±0.087	<0.1
l-noradrenaline + propranolol (1 μg/ml)	13	0.763±0.189	12	−0.272±0.123	<0.05	18	0.551±0.078	16	−0.442±0.103	<0.001
Propranolol (1 μg/ml)	12	1.020±0.203	11	−0.130±0.162	N.S.	12	1.088±0.137	10	0.041±0.095	N.S.
l-noradrenaline + phentolamine (5 μg/ml)	5	2.155±0.392	5	1.320±0.418	<0.05	4	2.713±0.408	4	2.066±0.303	<0.01
Phentolamine (5 μg/ml)	5	0.773±0.263	5	0.063±0.073	N.S.	4	1.281±0.385	4	0.634±0.361	N.S.
l-noradrenaline + phentolamine (50 μg/ml)	4	1.393±0.367	3	0.071±0.452	N.S.	5	2.712±0.279	5	1.403±0.264	<0.01
Phentolamine (50 μg/ml)	4	0.801±0.222	3	−0.430±0.263	N.S.	5	1.300±0.377	5	0.191±0.122	N.S.

[a] μmoles/g ww/2 h of incubation.
[b] The significance was tested from the paired difference between glycerol release in presence and absence of the different additions to the medium indicated in the Table.

duced markedly the lipolytic response to noradrenaline, to values significantly below the basal glycerol release of the tissue specimens in the both groups (−0.272±0.123 and −0.442±0.103 μmoles/g ww/2 h, $p<0.05$ and $p<0.001$ respectively) (Table II). Propranolol alone did not significantly modify the basal glycerol release.

Addition of the α-blocking agent phentolamine (5 μg/ml) enhanced the lipolytic action of noradrenaline in both types of tissue (Table II). This effect was most striking in tissue specimens from hypothyroid patients ($p<0.01$). Phentolamine (5 μg/ml) alone did not significantly affect the glycerol release. The use of a higher concentration of phentolamine (50 μg/ml) did not significantly stimulate the noradrenaline-induced lipolysis in the control group. However in the hypothyroid group a significant stimulation occurred also with the higher phentolamine concentration ($p<0.01$). Phentolamine alone (5 or 50 μg/ml) did not significantly change basal lipolysis in either group (Table II).

Effect of α-adrenergic stimulation on dibutyryl cyclic AMP and theophylline-induced lipolysis

Dibutyryl cyclic AMP induced a dose-dependent stimulation of glycerol output in tissue specimens

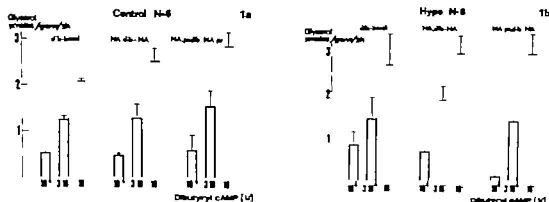


Fig. 1. Net lipolytic effect (mean ±S.E.M.) of different concentrations of dibutyryl cyclic AMP alone (db-basal) and in the presence of noradrenaline (NA, db-NA) or noradrenaline and propranolol (NA, pr db-NA, pr) in subcutaneous adipose tissue from controls (*a*) and hypothyroid subjects (*b*). The concentrations of dibutyryl cyclic AMP tested are shown. Concentrations of noradrenaline and propranolol 2 10^{-6} M and 1 μg/ml, respectively

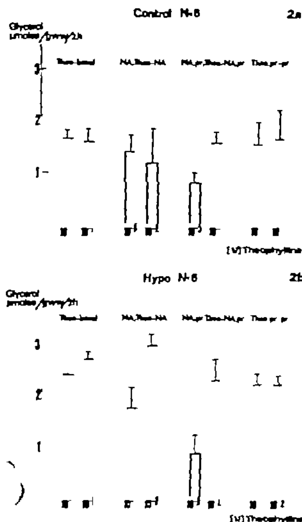


Fig. 2. Net lipolytic effect (mean ±S.E.M.) of tw concentrations of theophylline alone (Theo-basal) and in combination with noradrenaline (NA, Theo-NA), with noradrenaline plus propranolol (NA, pr Theo-NA, pr) or with propranolol alone (Theo, pr-pr) in subcutaneous adipose tissue from controls (*a*) and hypothyroid subjects (*b*). Noradrenaline and propranolol concentrations as in Fig. 1.

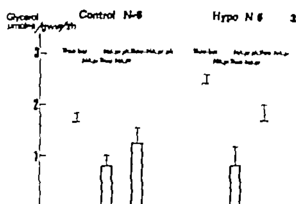


Fig. 3. Reversal of the inhibitory action of noradrenaline plus propranolol (NA, pr) on theophylline-induced lipolysis by phentolamine (ph, 40 µg/ml) in adipose tissue from controls and hypothyroid subjects. Concentrations of noradrenaline and propranolol as in Fig. 1. (Mean ±S.E.M.)

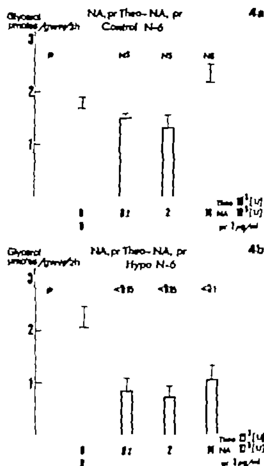


Fig. 4. Effect of three different concentrations of noradrenaline (NA) in the presence of propranolol (pr) on theophylline-induced (Theo) lipolysis (mean ±S.E.M.) in controls (*a*) and hypothyroid subjects (*b*). The *p*-values were calculated from the mean differences between the rate of lipolysis in the presence and absence of noradrenaline plus propranolol.

from both the control and the hypothyroid groups, the responses being of similar magnitude (Fig. 1). Noradrenaline, alone or in combination with propranolol, did not influence the lipolytic effect of dibutyryl cyclic AMP in either group.

In the presence of theophylline a marked increase in lipolysis was observed in tissues from both groups (Fig. 2). In neither group did the lipolytic effect of 10^{-3} or 10^{-2} M theophylline differ significantly. Lipolysis induced by the lower (10^{-3} M) concentration of theophylline was significantly inhibited by noradrenaline plus propranolol

in tissues from either controls ($p < 0.02$) or hypothyroid subjects ($p < 0.01$) while the effect of the higher theophylline concentration (10^{-2} M) was not influenced (Fig. 2). Noradrenaline alone had no significant effect on theophylline-induced (10^{-3} or 10^{-2} M) lipolysis in the control group. In the hypothyroid group there was a slight increase of the theophylline-induced (10^{-3} M) lipolysis by noradrenaline alone ($p < 0.1$), while the lipolytic effect of theophylline (10^{-2} M) was not significantly changed. The presence of propranolol alone did not modify the lipolytic response to theophylline in either group. The antilipolytic action of noradrenaline in combination with propranolol on theophylline-induced lipolysis could be partially blocked by the α-adrenergic antagonist phentolamine in both groups (p N.S. in control group and $p < 0.05$ in hypothyroid group) (Fig. 3). Glycerol release under these conditions was, however not significantly different from the release obtained with theophylline alone.

The antilipolytic action of three concentrations of noradrenaline (0.2, 2 or 14×10^{-3} M) in combination with propranolol (1 μg/ml) was tested on theophylline-induced (10^{-3} M) lipolysis in a separate group of subjects (Fig. 4). Inhibition could in these subjects be demonstrated only in the hypothyroid group: lipolysis was inhibited significantly by all the concentrations of noradrenaline used.

DISCUSSION

The data presented in this paper confirm an earlier report (18) on the inability of noradrenaline to induce lipolysis in adipose tissue from patients with hypothyroidism. It is generally accepted that the lipolytic effect of the catecholamines is mediated by the β-adrenergic receptor enhancing the formation of cyclic AMP which then stimulates the hormone-sensitive lipase (9, 11, 19). The addition of the almost pure β-adrenergic agonist isopropylnoradrenaline elicits a normal lipolytic response in tissue from hypothyroid subjects, indicating that the β-adrenergic receptor as such is not modified in this state (18). Furthermore, by the use of dibutyryl cyclic AMP which mimics the lipolytic effect of endogenously produced cyclic AMP it could be shown that the lipase system was not affected in hypothyroidism (Fig. 1). The unresponsiveness of the adipose tissue from hypothyroid subjects to noradrenaline could be related to an increase in the stimulation of the α-adrenergic receptors of the tissue, since the α-adrenergic antagonist phentolamine restored the lipolytic effect of noradrenaline (Table II).

The mechanism by which α-adrenergic agonists inhibit lipolysis is not known (17). In theory noradrenaline induced lipolysis may be suppressed by 1) inhibition of the stimulatory action of the hormone on adenyl cyclase, 2) stimulation of the enzyme phosphodiesterase which degrades cyclic AMP or 3) diminution of the responsiveness of the hormone sensitive lipase to cyclic AMP. In the present paper an attempt was made to clarify which of these alternative mechanisms was responsible for the antilipolytic action of α-adrenergic stimulation.

Stimulation of the α-adrenergic receptor in this study was obtained by using noradrenaline in combination with the β-adrenergic antagonist propranolol. The concentration of propranolol in the incubation medium was 1 μg/ml, a concentration shown to completely inhibit lipolysis induced by noradrenaline in normal omental adipose tissue (24). In subcutaneous adipose tissue from normal and hypothyroid subjects, the combination of noradrenaline and propranolol not only masked the lipolytic action of the catecholamine but resulted in a significant decrease of glycerol release below the basal level (Table II).

Since it is accepted that dibutyryl cyclic AMP acts by direct stimulation of the hormone-sensitive lipase (3) it was possible, by using this agent in combination with noradrenaline and propranolol, to clarify whether the antilipolytic effect of α-adrenergic stimulation was due to inhibition of the lipase system. The results demonstrated that stimulation of the α-receptors had no significant influence on the dibutyryl cyclic AMP induced lipolysis, even when lipolysis was not maximally stimulated (Fig. 1). These findings indicate that the antilipolytic action of the α-adrenergic receptors cannot be due to inhibition of the lipase system or its process of activation.

Theophylline a substance that prevents the degradation of endogenously produced cyclic AMP by inhibition of phosphodiesterase (7) was used to test whether the antilipolytic effect of α-adrenergic stimulation was mediated through inhibition of the formation of cyclic AMP. The results show that the combination of noradren-

aline and propranolol markedly reduced lipolysis induced by theophylline at the concentration of 10^{-3} M but not at 10^{-2} M in adipose tissue of hypothyroid subjects. This effect was less pronounced in tissue from control subjects and could not be demonstrated repeatedly By using different concentrations of noradrenaline it could again be shown that the inhibitory effect of the α-adrenergic receptor is more marked in adipose tissue from hypothyroid patients (Fig. 4) It is known that the levels of cyclic AMP that build up in the presence of high concentrations of phosphodiesterase inhibitors, by far exceed the concentration needed for maximal activation of the lipase system (6). Therefore, when using lipolysis as a measure of cyclic AMP concentration, changes taking place above the maximal cyclic AMP level needed for lipase activation would not be detected. In the present experiments no significant difference in the rate of lipolysis was observed using either 10^{-3} or 10^{-2} M of theophylline. Hence, the maximal level of cyclic AMP required for the activation of the lipase was probably obtained with the lower concentration of theophylline. This should explain the finding that only lipolysis induced by 10^{-3} M theophylline could be inhibited by noradrenaline in the presence of propranolol, but not that of ^{2} M of theophylline. These results do not the differentiation between the two pos- actions of α-adrenergic stimulation, decreased cyclic AMP formation or increased breakdown of cyclic AMP *Both effects would result in a net* reduction of the intracellular concentration of cyclic AMP and hence reduce lipolysis.

The results of the present study thus favour the hypothesis that the antilipolytic effect of α-adrenergic stimulation is elicited by decreasing the level of cyclic AMP in the adipocyte. Furthermore similar mechanisms seem to operate in adipose tissue from both euthyroid subjects and hypothyroid patients. The difference in noradrenaline response between the two groups is thus a quantitative rather than a qualitative one. In this context the results of Burns and Langley on isolated human adipose cells (4) are of interest. They were able to demonstrate that lipolysis induced by TSH was completely inhibited by the addition of adrenaline and propranolol to the medium. Furthermore Burns et al. (5) could show that α-adrenergic stimulation by adrenaline in the presence of propranolol reduced the cyclic AMP content of the cells to values below the basal level. These observations further support the hypothesis that the antilipolytic action of the α-adrenergic receptor is mediated through reduction of the intracellular level of cyclic AMP

In conclusion, agents like noradrenaline, which stimulate both the β- and α-adrenergic receptors in adipose tissue, possess lipolytic as well as antilipolytic properties. The net result of these effects on lipolysis will be controlled by the balance between the two receptor activities. The hypothyroid state favours the responsiveness of the α-receptors, thus reducing the lipolytic property of noradrenaline. A similar dual action of noradrenaline has been observed in frog skin preparations (1), isolated pancreatic islets (20) platelets (15) and the toad bladder (10) The effect of the hypothyroid state has so far not been tested on these systems.

The mechanism of action of thyroxin is still poorly understood. However several processes located at the cellular membrane seem to be influenced by thyroxine, and recent studies have revealed that the hormone can act without entering the cells (2) Some of the membrane functions that have been tested are glucose transport (23) ATP-ase activity (12) and adenyl cyclase activity (8) Any of these functions may be of importance in determining the α-adrenergic response to noradrenaline they might also account for the enhancement of the α-adrenergic response to noradrenaline in adipose tissue specimens from hypothyroid subjects described in this paper

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NORADRENALINE INDUCED LIPOLYSIS IN SUBCUTANEOUS ADIPOSE TISSUE FROM HYPOTHYROID SUBJECTS

The Relation of Noradrenaline Response to the Degree and Duration of the Disease

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Abstract. The lipolytic effect of noradrenaline in vitro is markedly reduced in subcutaneous adipose tissue from hypothyroid subjects. In earlier publications this was shown to be due to enhanced α-adrenergic receptor responsiveness. In the present study this phenomenon has been further investigated in 84 subjects with regard to the degree of hypothyroidism, the duration of the disease and the age and weight of the subjects. It could be shown that the hypothyroid state was the most important factor in determining the noradrenaline response. The impaired noradrenaline response was evident already after 1–2 months of thyroid hormone deficiency and was normalized with substitution therapy.

The lipolytic effect of noradrenaline in vitro is markedly reduced in subcutaneous adipose tissue from hypothyroid subjects. This impaired response could be attributed to a change in the balance between the α- and β-adrenergic receptors, induced by the hypothyroid state (12). Since addition of phentolamine, an α-adrenergic antagonist, could restore the lipolytic response to noradrenaline, it was concluded that the α-adrenergic response of the tissue was enhanced in the hypothyroid state. It could further be demonstrated that the α-receptor acted by lowering the level of cyclic AMP in the tissue (10).

The aim of the present study was to analyze the time course of the modification of noradrenaline response with the development of hypothyroidism, and to correlate the degree of defective response with the severity of the disease. Furthermore, the significance of other factors, such as body weight and age, on noradrenaline-induced lipolysis was evaluated.

METHODS

The clinical and laboratory findings in the 84 subjects included in this study are given in Table I. All except six persons had developed hypothyroidism after radioiodine treatment for thyrotoxicosis; five had had Hashimoto's disease and subsequently developed hypothyroidism; one (no. 80) had developed hypothyroidism after total thyroidectomy for cancer of the thyroid gland. The duration of the symptoms of hypothyroidism was estimated from the patients' records.

Thirty subjects were on replacement therapy with thyroid hormone (desiccated thyroid, Thyranon® or l-thyroxin, Levaxin®) and constituted the control group. The duration of full substitution therapy was calculated from the patients' records.

The ideal body weight percentage was calculated from standard tables (14).

Subcutaneous adipose tissue (3–5 g) was obtained from the thigh of non-fasting subjects by excision under local anesthesia, using 5–6 ml 0.5% prilocainchloride, Citanest® (Astra, Sweden). The fat tissue was transported in 0.9% NaCl at 37°C, and preincubated for one hour at 37°C in Krebs-Henseleit bicarbonate (KHB), pH 7.4, containing 1% bovine albumin. Separate tissue sections (50–80 mg) were then incubated for two hours at 37°C in 1.5 ml KHB (pH 7.4) containing 3% bovine albumin and 1 mg/ml glucose, as described earlier (12). The production of glycerol was used as an index of lipolysis. Glycerol in the incubation medium was analyzed spectrophotometrically (7, 16).

The diameters of the fat cells were analyzed on frozen sections of the tissue according to Sjöström et al. (13).

Due to the limited amount of fat obtained by the biopsy technique, all experiments could not be performed on specimens from each subject.

Determinations of PBI, cholesterol, resin uptake of ^{125}I-labeled triiodothyronine (T_3) and thyroid uptake of radioiodine at 4 hours were performed in non-fasting patients as previously described (12), and the same values for the normal ranges were used.

Table I. *Clinical and laboratory findings in the control, borderline and hypothyroid groups*

Pat. no.	Age (y.)	Sex	% of ideal b.wt.	PBI (µg/100 ml)	Cholesterol (mg/100 ml)	Resin uptake of T_3-^{125}I (%)	TSH (µU/ml)	Thyroidal 24-hour uptake of ^{131}I (%)	Treatment[a] (mg/d)	Duration of treatment or hypothyroidism (y.)	(mo.)
Control group											
1	44	♀	—	4.6	315	30	—		T 112.5	3	0
2	47	♀	119	8.2	248	32	<12.5		L 0.3	0	2
3	48	♀	90	9.2	341	32	<12.5		L 0.25	0	4
4[b]	49	♀	98	12.7	288	27	18		F 75	1	0
5	49	♀	117	5.8	267	37	<12.5		L 0.2	0	2
6	50	♀	82	4.8	271	25	—		T 75	6	0
7	52	♂	79	5.4	253	37	<12.5		T 112.5	0	3.5
8	54	♀	97	5.0	268	—	—		T 75	9	10
9	55	♀	92	4.2	330	28	28		T 75	4	0
10	55	♀	92	6.0	289	31	—		L 0.15	0	1.5
11	56	♀	131	4.8	291	31	<12.5		T 225	0	3
12	57	♀	129	7.8	295	33	<12.5		L 0.2	0	6
13	58	♀	—	>30[d]	331	28	—		L 0.2	0	7
14	59	♀	88	8.7	300	31	<12.5		L 0.15	0	3
15	60	♀	97	7.0	283	26	<12.5		L 0.2	0	11
16	61	♂	102	3.9	317	28	—		T 75	0	2
17	62	♀	98	7.3	260	25	<12.5		L 0.15	0	7
18	63	♀	—	14.0	206	16	—		T 112.5	6	0
19	64	♀	97	8.2	349	28	<12.5		L 0.15	0	3
20	65	♀	99	5.9	255	28	28.5		T 112.5	0	2.5
21	66	♀	—	3.9	324	31	—		T 56.2	9	0
22[b]	67	♀	88	9.8	211	33	17.3		L 0.2	0	3
23	67	♀	91	6.4	252	30	<12.5		T 112.5	0	5
24[b]	68	♀	95	11.4	244	36	14		L 0.15	0	6
25	68	♀	99	7.3	258	26	22.6		T 112.5	0	6
26	68	♀	133	12.5	247	29	24.5		L 0.2	0	2
27	69	♂	103	5.0	264	28	—		T 112.5	3	0
	71	♀	137	5.8	286	—	<12.5		T 150	1	0
	75	♀	98	8.3	200	31	<12.5		L 0.15	0	4
	75	♀	115	8.2	198	27	—		L 0.2	0	4
-derline group											
31	51	♀	93	5.5	335	23	35	18			
32	57	♂	100	4.9	233	29	27.5	20			
33	57	♂	112	3.7	255	25	25.5	12			
34	63	♂	111	3.7	371	27	27.5	26			
35	64	♀	98	4.6	420	24	21	16			
Hypothyroid group											
36	37	♀	162	2.5	427	17	87	9		3.5	0
37	41	♂	91	3.6	224	33	118	27		0	6
38	44	♀	98	2.2	313	23	120	3.5		6	0
39	46	♀	91	5.1	255	20	128	16		0	3
40	47	♀	85	2.6	300	22	100	15		0	2
41	47	♀	118	1.6	378	17	138	6		0	3
42	48	♀	96	9.7[e]	536	21	—	1		0	1.5
43	51	♀	—	2.7	386	22	195	1		0	1.5
44[b]	51	♀	80	—	296	—	49	—		0	5
45	51	♀	96	3.6	560	4	77	5		0	1
46[b]	51	♂	96	1.9	319	27	196	26		1	0
47	52	♀	85	3.9	412	28	—	27		0	6
48	52	♀	118	3.2	406	24	—	28		0	3
49	54	♀	102	2.3	463	25	—	—		0	1
50	55	♀	96	2.8	504	24	105	25		0	6
51	55	♀	—	2.7	441	27	—	21		0	1
52	55	♀	133	2.7	375	27	80	20		0	3.5
53	56	♀	98	3.7	283	21	105	29		0	2.5
54	56	♀	128	4.8	308	29	43	8.4		0	4
55	56	♀	135	3.2	485	27	134	7		2	0
56	57	♀	135	4.1	456	21	115	12		1	0

Table I (*continued*)

Pat. no.	Age (y)	Sex	% of ideal b wt.	PBI (μg/100 ml)	Cholesterol (mg/100 ml)	Resin uptake of T_3–^{125}I (%)	TSH (μU/ml)	Thyroidal 24-hour uptake of ^{131}I (%)	Treatment[a] (mg/d)	Duration of treatment or hypothyroidism (y.)	Duration of treatment or hypothyroidism (mo.)
57	58	♀	107	4.1	338	26	43	17		0	>2
58	59	♀	90	3.2	355	26	189.4	16		0	4
59	59	♀	99	3.0	476	20	75	14		0	6
60	59	♂	113	<2.5	390	24	98	2		0	1.5
61	59	♀	118	2.9	289	26	88	20		0	3
62	60	♀	89	2.9	506	23	200	14		0	>1.5
63	61	♀	—	3.0	376	27	—	17		0	4
64	61	♀	99	3.8	351	20	70	13		0	4
65	61	♂	114	<2.5	418	23	118	7		0	2
66	63	♀	85	<2.5	618	21	100	11		0	2
67	63	♀	93	<2.5	290	26	—	6		0	6
68	63	♂	116	3.5	262	20	103	11		1	0
69	64	♀	108	5.3	310	29	135	14		0	1.5
70	64	♀	129	4.8	486	23	—	23		0	4
71	65	♀	105	6.2	211	23	63	27		0	6
72[b]	66	♂	116	<2.0	637	23	171.6	4		>2	0
73	66	♀	119	3.4	372	22	71	7		0	4
74	68	♀	116	4.1	371	4	—	14		0	1.5
75	68	♀	101	3.3	365	26	87.5	—		0	1
76	68	♀	136	4.8	297	29	135	21		0	4
77	70	♀	—	3.4	341	24	—	20		0	2
78	71	♀	76	8.7[c]	262	17	90	—		1	0
79	72	♀	102	4.1	339	25	100	—		0	7
80	72	♀	135	1.9	489	24	175	3.5		0	4
81	74	♀	105	<2.5	343	24	68	1		0	2
82	74	♀	130	1.8	338	26	105	—		1	0
83	76	♀	84	<2.5	436	19	—	8		0	1
84	79	♀	104	[illegible]	327	28	—	19		0	2

L = Levaxin® T = Thyranon®
[b] Hashimoto's disease.
High value probably due to iodine contamination.

TSH was measured by the double antibody technique described by Odell et al. (9). The normal range in our laboratory was 12.5–40 μIU/ml. Lower values than 12.5 μU could not be measured. The S.D. was ±31% of the mean.

The following pharmacological agents were used in the incubations: l-noradrenaline bitartrate (Astra, Sweden), phentolamine, Regitine® (Ciba), N⁶ 2'O-dibutyryl-3 5 adenosine monophosphate (dibutyryl cyclic AMP) (Boehringer/Mannheim, West Germany), propranolol, Inderal® (ICI, England). Theophylline was obtained commercially.

Due to an error in the calculations the final concentrations of noradrenaline given in previous publications (11, 12) are too high. The concentrations should be divided by factor of 1.42. This correction has been made in the present publication.

Stepwise multiple regression analysis (4) was performed by use of BMD programs (2) and on IBM 360/75 computer.

RESULTS

The subjects studied are presented in Table I (all results in this paper are presented as mean values ±S.E.M.). The distribution of age and ideal body weight percentage did not differ between the two groups (Fig. 1). The age was 60.1±1.6 years in the control group and 59.6±*1.3 years in the hypothyroid group. The ideal* body weight percentage was 107.4±2.8 and 102.5±3.1 in the control and hypothyroid groups, respectively. Neither did age or ideal body weight percentage differ between the two groups when tested with a one way analysis of variance (8).

The duration of substitution therapy in the control group varied between *1.5 and 118 months.*

The duration of hypothyroidism, as estimated from the patients' records, varied considerably (Table I). The 35 hypothyroid subjects tested with TSH were divided into four subgroups with respect to the duration of the disease (1–2, 3–4, 5–11 and 12–27 months). The number of subjects in each subgroup was 10, 10, 6 and 9, respectively.

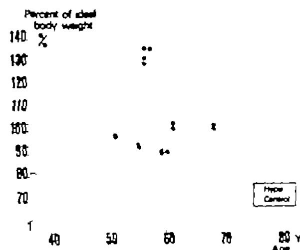


Fig. 1 Relationship between the ideal body weight percentage and age in the control and hypothyroid groups.

A small group of patients, whose TSH values were below 40 μU without substitution therapy constituted the borderline group. These subjects were not included in the control group since they were regarded as mildly hypothyroid in clinical groups. Lipolytic data from these subjects were only used for the study of the relationship between the noradrenaline response and the values of TSH PBI, resin uptake of T_3 and cholesterol. One of these subjects was also included in the group of patients studied before and after treatment with thyroid hormones.

The basal rate of lipolysis was similar (1.011 ± 0.083 and 0.991 ± 0.078 μmoles/g ww/2 h) in the control and hypothyroid groups (Table II). The lipolytic response to noradrenaline (2×10^{-6} M) differed markedly between the two groups (Table II). In the control group noradrenaline stimulated lipolysis significantly ($p < 0.001$) while no such stimulation occurred in the hypothyroid group. The addition of phentolamine (0.5 5 or 50 μg/ml) increased noradrenaline-induced lipolysis in the control group the lowest dose (0.5 μg) being most effective. In the hypothyroid group the α-adrenergic blocker restored the lipolytic action of noradrenaline In this tissue the higher doses of phentolamine seemed to be more effective. Phentolamine alone did not change the rate of basal glycerol release in either group (Table II). Isopropylnoradrenaline (3×10^{-8} M) theophylline (10^{-3} or 10^{-2} M) and dibutyryl cyclic AMP (10^{-3} M) stimulated lipolysis significantly in both groups (Table II).

In a different group of subjects a dose-dependent stimulation of noradrenaline-induced lipolysis could be demonstrated in the control group while tissues from the hypothyroid subjects did not respond even to very high concentrations of the

II *Effect of different lipolytic agents and an α-adrenergic antagonist on glycerol release from subcutaneous adipose tissue from control and hypothyroid subjects*

	Control group				Hypothyroid group			
Addition to medium	N	Mean ± S.E.M. (μmoles/g ww/2 h)	Mean diff ± S.E.M.	p^a	N	Mean ± S.E.M. (μmoles/g ww/2 h)	Mean diff ± S.E.M.	p^a
None	30	1.011 ± 0.083	—	—	48	0.991 ± 0.078	—	—
l-noradrenaline (2×10^{-6} M)	30	1.711 ± 0.139	0.707 ± 0.101	< 0.001	48	0.864 ± 0.080	−0.129 ± 0.080	N.S.
l-noradrenaline (2×10^{-6} M) + phentolamine (0.5 μg/ml)	6	3.083 ± 0.200	2.196 ± 0.184	< 0.001	9	2.071 ± 0.357	1.010 ± 0.304	< 0.0-
l-noradrenaline (2×10^{-6} M) + phentolamine (5.0 μg/ml)	5	2.156 ± 0.393	1.320 ± 0.418	< 0.05	13	2.157 ± 0.263	1.293 ± 0.251	< 0.001
l-noradrenaline (2×10^{-6} M) + phentolamine (50 μg/ml)	14	2.081 ± 0.161	1.062 ± 0.161	< 0.001	24	2.252 ± 0.131	1.250 ± 0.111	< 0.001
Phentolamine (0.5 μg/ml)	—	—	—	—	2	1.170 ± 0.046	−0.259 ± 0.106	N.S.
Phentolamine (5.0 μg/ml)	5	0.773 ± 0.263	−0.063 ± 0.075	N.S.	13	1.042 ± 0.174	0.178 ± 0.150	N.S.
Phentolamine (50 μg/ml)	4	0.931 ± 0.343	−0.221 ± 0.263	N.S.	8	1.309 ± 0.229	0.164 ± 0.098	N.S.
l-isopropylnoradrenaline (3×10^{-8} M)	21	3.103 ± 0.267	2.030 ± 0.284	< 0.001	27	2.844 ± 0.184	1.801 ± 0.171	0.001
Dibutyryl cyclic AMP (10^{-3} M)	14	3.068 ± 0.203	2.228 ± 0.190	< 0.001	20	3.052 ± 0.226	2.041 ± 0.224	< 0.001
Theophylline (10^{-3} M)	13	2.996 ± 0.177	1.855 ± 0.219	0.001	19	3.108 ± 0.258	2.136 ± 0.183	< 0.001

The significance was tested from the paired difference between glycerol release in presence and absence of the different additions to the medium.

Table III. *Basal and l-noradrenaline* (*NA*) (2 × 10^{-6} M) *stimulated lipolysis in 14 subjects before and after thyroid hormone treatment*

Pat. no. in Table I		Glycerol release (μmoles/g ww/2 h) Type of lipolysis: Basal			NA-basal		
Before	After	Before	After	Diff.	Before	After	Diff.
41	2	2.605	1.478	−1.127	−1.608	1.007	2.615
81	29	1.094	0.951	−0.143	0.027	1.428	1.401
50	10	0.996	0.715	−0.281	−0.048	1.239	1.287
76	26	1.976	2.095	0.119	−0.087	0.956	1.043
75	24	1.057	1.065	0.548	−0.059	0.860	0.919
53	12	0.706	1.017	0.311	0.044	0.853	0.809
50	11	0.988	1.585	0.597	−0.218	0.521	0.739
41	4	1.324	1.132	−0.192	0.544	1.237	0.653
57	14	0.717	0.071	−0.646	−0.011	0.472	0.483
82	30	0.841	1.481	0.640	1.230	0.750	0.480
40	3	0.757	1.318	0.561	−0.220	0.236	0.456
63	17	0.386	0.456	0.070	−0.051	0.253	0.304
35	19	0.147	0.919	0.772	−0.007	0.115	0.122
74	25	1.040	1.303	0.236	0.305	−0.693	−0.998
Mean		1.045	1.152	0.107	0.009	0.660	0.668
±S.E.M.		0.165	0.139	0.145	0.160	0.150	0.229
p				N.S.	N.S.	<0.005	<0.02

amine (Fig. 2). A slight inhibition of lipolysis was observed at the noradrenaline concentration of 2 × 10^{-8} M. The addition of phentolamine (50 μg/ml) markedly stimulated lipolysis in the hypothyroid patients but to a much lesser degree in the controls (Fig. 2).

In 14 subjects the lipolytic response to noradrenaline was studied before and after replacement therapy (Table III). The treatment resulted in a significant increase in the lipolytic response to noradrenaline ($p < 0.02$). The basal glycerol release, however, was not significantly modified.

There was no relationship between the noradrenaline (2 × 10^{-6} M) response and the duration of full thyroid replacement therapy within the time limits studied (1.5 and 118 months): the equation of the regression line being $y = 0.00015X + 0.704$ (correlation coefficient = 0.009 N.S.).

The lipolytic response to noradrenaline (2 × 10^{-5} M) in adipose tissue from hypothyroid subjects was markedly inhibited already after 1–3 months of disease and no further change could be observed (Table IV). Since the duration of the hypothyroid state had to be estimated retrospectively from the patients' records, the time periods indicated in Tables I and IV have to be regarded as an approximation of real duration.

The relationship between the noradrenaline (2 × 10^{-6} M) response and the values of PBI, resin uptake of T_3, cholesterol, TSH and thyroid uptake of radioiodine at 24 hours is presented in Figs. 3–7. Only weak correlations within the hypothyroid group were found using these parameters alone or in combination (Table V).

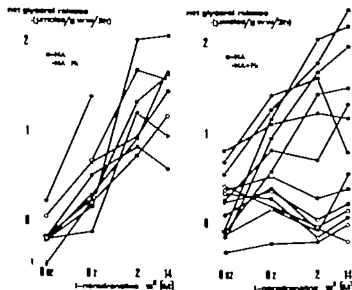


Fig. 2. Lipolytic effect of increasing concentrations of l-noradrenaline alone or in combination with phentolamine (50 μg/ml) on subcutaneous adipose tissue from control (left) and hypothyroid subjects (right).

Table IV *Significance of duration of hypothyroid symptoms for serum TSH level, basal glycerol release and response to l-noradrenaline (NA) (2 10^{-6} M) in subcutaneous adipose tissue in vitro (mean ±S.E.M)*

Duration (mo)	N	TSH (μU/ml)	Glycerol release (μmoles/g ww/2 h) Basal	NA	NA-basal	p*
1 2	10	126.6±15.2	0.919±0.147	0.849±0.167	−0.070±0.143	N.S.
3–4	10	100.3±14.7	1.262±0.204	0.850±0.155	−0.413±0.151	<0.025
5–11	6	85.0±10.9	1.166±0.129	1.003±0.148	−0.163±0.073	<0.1
12 72	9	124.6±12.3	0.848±0.201	0.629±0.247	−0.219±0.254	N.S.

* Significance of difference in lipolysis in presence and absence of noradrenaline.

Table V *Result of the stepwise multiple regression analysis of the relationship between the noradrenaline response in subcutaneous adipose tissue from hypothyroid subjects and different laboratory tests of thyroid functions*

The test was made with the values of 29 patients in whom all the tests had been performed. Values denoted with the signs > or < were used with the value of the limit

Variables used in the analysis	Variable no.	Correlation coefficient	p
Resin uptake of T_3–^{125}I (%)	1	0.414	<0.05
Log PBI (μg/100 ml)	2	0.356	N.S.
PBI (μg/100 ml)	3	0.321	N.S.
TSH (μU/ml)	4	0.144	N.S.
24-h uptake of ^{131}I (%)	5	0.141	N.S.
Log cholesterol (mg/100 ml)	6	−0.069	N.S.
Cholesterol (mg/100 ml)	7	−0.014	N.S.
2		0.481	<0.05
2+4		0.542	<0.05
2+4+6		0.575	<0.05
1+2+4+6+5		0.583	N.S.

No significant relationship was found between the noradrenaline response and percentage of ideal body weight in either the control or hypothyroid group ($r = 0.186$ and 0.189 respectively).

The diameter of the adipose cells was measured in tissue specimens from six controls and six hypothyroid subjects (Table VI). The diameter was 108.0±11.6 and 94.6±8.4 μ in the control and hypothyroid groups, the difference being non-significant. However the corresponding noradrenaline responses differed significantly between the two groups ($p < 0.005$ Table VI).

DISCUSSION

The hypothyroid state in man is accompanied by a number of clinical signs and symptoms, the intensity of which seems to be correlated with the degree of thyroid hormone deficiency and the duration of disease (5) Some of the deranged functions are rapidly normalized by thyroid hormone replacement while others need longer periods of treatment in order to be eliminated. Thus, secretion of TSH (15) or glomerular filtration rate (1) is normalized within a short time while, e.g. the rate of free water reabsorption in the kidney responds slowly to treatment (1) No single biochemical parameter measures adequately the degree of thyroid hormone deficiency in different cells of the body

It was therefore of interest to investigate how

Table VI *Fat cell size and noradrenaline-induced (2 10^{-6} M) lipolysis in vitro in control and hypothyroid subjects*

	Control group (N=6) Mean±S.E.M.	Mean diff ±S.E.M.	p*	Hypothyroid group (N=6) Mean±S.E.M.	Mean diff ±S.E.M	p*	p†
Mean cell diameter (μ)	108.0±11.6	—	—	94.6±8.4	—	—	N.S.
Glycerol release (μmoles/g ww/2 h)							
Basal	1.271±0.281	—	—	1.066±0.154	—	—	
Noradrenaline	2.104±0.421	0.833±0.209	<0.02	0.658±0.166	−0.408±0.208	N.S.	<0.005

* Significance of difference in lipolysis in presence and absence of noradrenaline.
† Significance of difference between the control and hypothyroid groups.

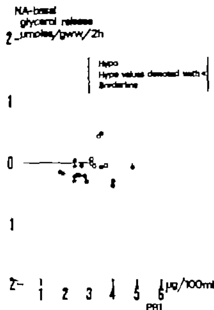


Fig. 3. Relationship between the net noradrenaline response (NA-basal) in subcutaneous adipose tissue from hypothyroid patients and their PBI values. The values of the borderline group have also been plotted. PBI values denoted with the sign < are specially marked.

the noradrenaline unresponsiveness of subcutaneous adipose tissue from hypothyroid subjects, described earlier (12) would correlate on the one hand with the different biochemical parameters used for the evaluation of the thyroid hormone

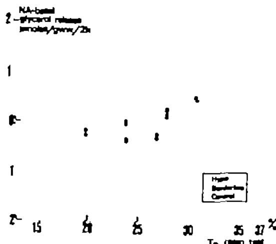


Fig. 4. Relationship between the net noradrenaline response (NA-basal) in subcutaneous adipose tissue from hypothyroid subjects and the values of resin uptake of T (T_3 resin test) in control, borderline and hypothyroid groups.

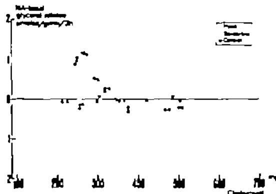


Fig. 5. Relationship between the net noradrenaline response in subcutaneous adipose tissue from the hypothyroid patients and their values for cholesterol in control, borderline and hypothyroid groups.

status, and with the duration of the disease on the other.

It is shown in Table IV that the lipolytic response to noradrenaline was markedly reduced already after 1 $^1/_2$–2 months of disease. Furthermore, even a small increase in TSH in the borderline group seemed to be accompanied by a reduced noradrenaline response (Fig. 6). These findings would indicate that the impaired noradrenaline response is an early sign of the disease and oc

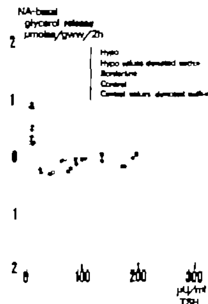


Fig. 6. Relationship between the net noradrenaline response in subcutaneous adipose tissue and the TSH level of the control, borderline and hypothyroid groups. TSH values denoted with the sign < have been marked specially.

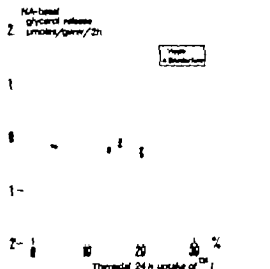


Fig 7 Relationship between the net noradrenaline response in subcutaneous adipose tissue and the thyroidal 4-hour uptak of ¹³¹I of the patients in the hypothyroid group.

curs already with a small decrease in thyroid hormone concentration at the adipose tissue level. This could also explain the weak correlation between the biochemical parameters and the noradrenaline response. Probably none of the tests reflect the actual concentration of thyroid hormones in adipose tissue

The findings presented in this report on a large oup of hypothyroid patients confirm our pre ous reports (10 11 12) on the reduction of noradrenaline-induced lipolysis in this disease. In the control group there was a dose-related increase of glycerol release to noradrenaline stimulation whereas even high concentrations of the amine were unable to stimulate lipolysis in adipose tissue specimens from hypothyroid subjects. The addition of an α-adrenergic antagonist, phentolamine markedly stimulated the lipolytic response to noradrenaline in tissue specimens from the hypothyroid subjects, while a much smaller increase was observed in the control group. The absence of lipolytic response to noradrenaline in hypothyroidism has previously been explained on the basis of an enhancement of the α-agonistic action of the catecholamine. The dose–response relationships presented here confirm this interpretation and exclude the possibility of an increased destruction or re-uptake of noradrenaline in the nerve endings in this tissue as being the cause of diminished lipolysis since phentolamine in the lower concentrations used here does not block the uptake mechanisms of the nerve endings (6).

The lipolytic response to isopropylnoradrenaline dibutyryl cyclic AMP and theophylline were of the same magnitude in both groups (Table II) These results justify the interpretation that decreased lipolysis in hypothyroidism is mainly due to enhancement of α-adrenergic response to noradrenaline, rather than to impairment in the β-adrenergic receptor—adenyl cyclase—lipase system of the adipocyte (12).

Our previous data were based on observations made on different subjects in the hypothyroid and control groups. In a smaller group of patients, studied both before and after substitution therapy the same changes in noradrenaline response were observed (Table III) This would indicate that the phenomenon described here cannot be due to the fact that different subjects were studied in the two groups but must be related to the hypothyroid state per se.

It is known that the lipolytic response to catecholamines, theophylline and dibutyryl cyclic AMP is reduced in adipose tissue from obese subjects (17) Therefore it was of interest to see whether the hypothyroid subjects were more overweight than the controls. As demonstrated in Fig. 1 the weight and age distribution was similar in the two groups. Furthermore, increasing weight exceeding the ideal b.wt. was not accompanied by a reduced lipolytic response to noradrenaline in either group. There was no significant difference in fat cell diameter between the two groups (Table IV) The number of female subjects by far exceeded that of males in both the control (2/28) and the hypothyroid (7/45) groups. However the sex difference has probably a minor influence on the lipolytic response as shown on human omental adipose tissue in vitro (4), and no attempt was made to separate the groups with regard to sex. All these findings indicate clearly that the differences in lipolytic response between controls and hypothyroid patients were not due to changes in parameters correlated with body weight, age or sex, but they must be related to the hypothyroid state as such.

ACKNOWLEDGEMENT

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Dopamet

l α metyldopa

Om Andersson behöver	½ + ½	= 1 tabl
Petersson „	1½ + 1	= 2½ tabl
Lundström	2 + 2	= 4 tabl
		= 7½ 3 = 2½

och genomsnittsdosen alltså blir 2½ tabl kan man då säga att det bara är Petersson som är välanpassad?
Vad säger Andersson och Lundström om det?
Ingenting – dom har redan talat med sin läkare

dosera individuellt
dosera 2 gånger per dag
utnyttja delbarheten

DUMEX

Acta med. scand. Vol. 192, pp. 371–376, 1972

DOUBLE BLIND TRIAL WITH INCREASING DOSES OF SALBUTAMOL AND TERBUTALINE AEROSOLS IN PATIENTS WITH REVERSIBLE AIRWAYS OBSTRUCTION

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Abstract. In a double-blind trial we have tested the effects of inhaling increasing doses of salbutamol and terbutaline aerosol. In 12 patients with reversible airways obstruction we assessed the effects on the airways obstruction by dynamic spirometry and repeated measurements of peak expiratory flow rate (PEFR) over a period of 5 hours after inhalation of 0.10+0.20 mg salbutamol and 0.25+0.50 mg terbutaline. A similar and significant bronchodilation occurred up to 2 hours, with 30% maximum increase after 1 hour. After 3–4 hours the effect declined, but was still significant. The ventilatory effect of terbutaline was significantly larger than that of salbutamol after 3–5 hours. No consistent significant changes or differences between the drugs were noted concerning pulse rate, BP or subjective side-effects.

The introduction of long-acting β-2-stimulating sympathomimetic agents has increased the possibility to improve bronchospasmolytic therapy in obstructive airways disease. Due to the more specific effect on receptors in the bronchial muscles and the lesser side-effects on the circulation, there seems to be a larger safety margin with the new type of agent. It has been shown, however, that, given per os or parenterally these agents can *still cause considerable side-effects*, especially muscular tremor. The use of aerosol, therefore, seems to be the most promising way to administer the drugs.

The present study was designed to compare bronchodilatory and circulatory effects after inhalation of increasing aerosol doses of salbutamol (Ventoline, Glaxo) and terbutaline (Bricanyl®, Astra) from the commercially available nebulizers.

PATIENT MATERIAL

We tested 12 patients with partially reversible airways obstruction in a relatively stable phase. Their average age was 51 years (27–67). Anthropometrical, clinical and spirometrical data are given in Tables I, II and III.

METHODS AND PROCEDURE

The patients were admitted to the Lung Clinic and kept on the same medication schedule during the trials. No bronchodilators were given later than 6 hours before the start of the studies each day. The patients were not allowed to have coffee or tea or to smoke during the time before or during the tests.

The patients performed dynamic spirometry (FEV, VC) on a Bernstein type spirometer and peak expiratory flow rate measurements (PEFR) with a Wright Peak Flow Meter (18). The data obtained were related to normal values according to Berglund et al. (3), and Vale (16). All studies were conducted by the same technician. Three spirograms and PEFRs were always recorded and the highest value was used for the statistical evaluation. Basal spirometric data will be seen in Table III.

To make a double-blind trial with the two commercially available inhalers we used two nebulizers at each administration. One nebulizer was used exactly as it came from the manufacturers, the other nebulizer contained a placebo but had the outward appearance of

Table I. *Physical data and diagnoses*

Subject no	Sex	Age (y.)	Height (cm)	Weight (kg)	Diagnoses
1	♂	27	177	75	Asthma + sarcoidosis?
2	♂	61	169	61	Asthma
3	♂	43	170	79	Asthma
4	♂	62	172	91	Asthma + emphysema
5	♂	43	183	80	Asthma
6	♀	31	156	52	Asthma
7	♂	63	192	88	Chr. bronchitis
8	♂	53	175	80	Asthma. Chr. bronchitis
9	♂	67	172	78	Chr. bronchitis + Br. ectases (op.)
10	♂	56	178	75	Asthma
11	♂	51	172	98	Asthma
12	♂	56	169	49	Asthma

Table II. *Clinical data*

Subject no.	Duration of dis. (y.)	Allergy: Hered.	Allergy: Personal (ic tests)	Eosinophils (%)	Smoking: y	Smoking: cig/day	Chest X-ray
1	1	0	0	15	5	5–10	Scattered micronodul.
2	20	0	+	3[a]	10	1 2	Hyp. infl.
3	1	0	0	15	0		Normal
4	50	+	+	5[a]	0		Hyp. infl.
5	8	0	0	12	0		Hyp. infl.
6	35	+	+	3[a]	0		Hyp. infl.
7	30	0	0	5	42	20	Hyp. infl.
8	8	0	+	6	35	1–2	Hyp. infl.
9	60	0	0	0	33[b]	2 pk/w	Pleur. thick. dx.
10	2	+	+	21	8	12	Normal
11	12	+	+	7.5	40	6–15	Normal
12	17	+	+	2[a]	15	10	Hyp. infl.

[a] On steroids. [b] Finished smoking

the alternate drug being tested. The nebulizers were numbered and matched in advance, but their contents were unknown to the patient and the technician until the code was broken at the end of the 12 test series. The tests were randomized, thus the patient inhaled terbutaline 0.25 mg + 0.50 mg and salbutamol 0.1 mg + 0.2 mg in random order during the two test days. The variation between the basal PEFR values during those two days was not allowed to be more than 15

Only patients, whose PEFR increased by 20% or more of the basal maximum PEFR value after inhalation of 0.16 mg isoproterenol on the first day were selected for further comparison between salbutamol and terbuta-

The experiment periods started at 10 o clock in the morning, usually on three successive days. The comparison started with measurements of pulse rate and BP and thereafter the patient was subjected to peak flow measurements and spirometry. The patients then inhaled one puff from the two nebulizers, one with drug (i.e. 0.25 mg terbutaline or 0.1 mg salbutamol), one with placebo, and the effect on airways obstruction was assessed with three measurements of PEFR 5 and 30 min after the first inhalation period. Thirty minutes after the first inhalation the patient took two more inhalations (i.e. 0.50 mg terbutaline or 0.. mg salbutamol) from each of the same two nebulizers as were used at the first single inhalation. PEFR was then measured 5, 30, 60, 120, 180, 40 and 300 min after the second inhalation. Spirometry was performed each day before drug administration and at the end of the tests 300 min after the second two aerosol inhalations.

RESULTS

There was no significant difference between pairs of individual control values for PEFR from the first day (i.e. with the evaluation of reversibility

Table III. *Control spirometry and PEFR of inhalation of 0.16 mg isoproterenol*

Subject no.	VC (pred)	FEV pred	FEV after isopr	FEV Found	FEV Normal (2 S.D.)	PEFR pred.	PEFR after isopr
1	63	28	31	37	68	60	38
2	84	64	20	53	56	49	43
3	50	45	6	68	62	54	30
4	49	34	45	48	55	31	37
5	70	53	22	59	61	66	34
6	86	55	24	55	73	31	36
7	60	33	35	38	55	42	60
8	42	25	2	43	58	41	27
9	51	47	7	61	53	38	21
10	53	28	20	37	57	28	34
11	69	53	5	56	59	63	1
12	70	39	15	41	58	40	20
Mean	62	42	19	49	59	45	37

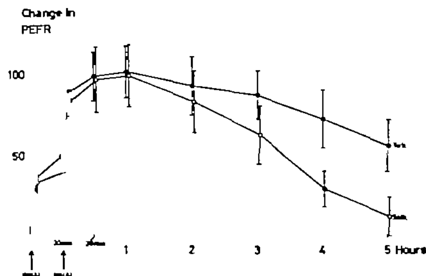


Fig. 1. Increase in PEFR (l/min) after inhalations of salbutamol and terbutaline.

with isoproterenol) and the days for the tests of salbutamol or terbutaline.

The mean values for PEFR and pulse rate are shown in Table III and Figs. 1, 2 and 3. The average control PEFR in the two series on the days for tests of salbutamol and terbutaline did not differ significantly (11 l/min).

The mean PEFR increased by 37% (20–86%) 5–10 min after inhalations of 0.16 mg isoproterenol (Table III). A significant bronchodilation in the form of an increased PEFR occurred with both salbutamol and terbutaline 5 min after the first single inhalation (+16–19%). After two further inhalations of the active drug the maximum effect on PEFR (+48%) was encountered after one hour. The bronchodilatory effect of both drugs was still significant after 4 hours. Terbutaline had a larger lasting effect than sal-

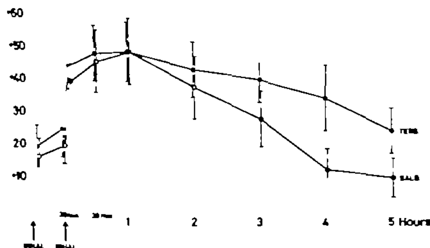


Fig. 2. Increase in PEFR (% of pre-drug values) after inhalations of salbutamol and terbutaline.

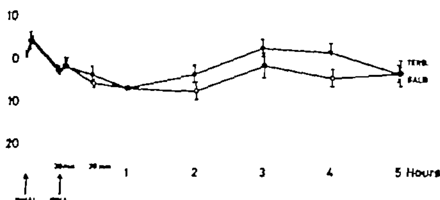


Fig 3 Change in pulse rate during five hours after inhalations of salbutamol and terbutaline.

butamol, showing up as significantly higher PEFR values 3 4 and especially 5 hours after the second inhalations.

The FEV_1 and VC increased significantly from the control period to measurements made 5 hours after the second two inhalations with on average 0.3 and 0.35 l after terbutaline and with 0.1 and 0.2 l, respectively after salbutamol. The value of FEV_1 after 5 hours was significantly higher after terbutaline than after salbutamol.

Pulse rate There was no significant difference between the control pulse rates in the two series. The mean values were around 80 beats/min. There was a significant decrease in pulse rates after 1–2 hours after three doses, but no consistent difference between the two drugs (Fig. 3).

ECG changes. ECG was recorded before and 30 min after the first single inhalation and 30 min after the second two inhalations. In the control state two subjects showed extrasystoles, two had S-T T depressions, one due to digitalis; one patient had a slight RBBB After inhalation

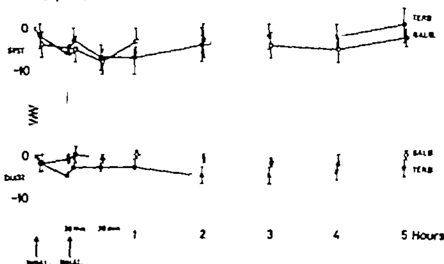


Fig 4 Change in systolic and diastolic BP during five hours after inhalations of salbutamol and terbutaline.

of the drugs there was no arrhythmia and no lowering of T waves or S-T segments; rather the T waves had a tendency to become larger with a lowering of the heart rate after the control ECG

Blood pressure There was no consistent significant effect on systolic or diastolic BP in the two series, the direction of change being toward a decrease of pressure (Fig. 4)

Subjective effects

The subjective discomforts from airways obstruction were initially graded as none in 2, slight in 8 and severe in 2 subjects in both test series.

Thirty minutes after the first single inhalation of salbutamol 5 patients thought they were "unchanged" 7 "better" After terbutaline 3 claimed to be unchanged and 9 better one complained of some vertigo.

Thirty minutes after the second two inhalations of salbutamol 4 patients felt unchanged, 4 better and 2 much better 1 had some vertigo. After terbutaline 8 claimed to be unchanged, 2 better and 1 much better

DISCUSSION

In the present study we selected patients with significant reversibility who were tested at comparable basal values of airways obstruction, which is necessary in order to evaluate the effects of two drugs on different days (11 14). The simultaneously given doses of the two drugs were comparable at each inhalation and were doubled during each test run to ensure a dose-response relationship.

Tests of bronchodilating activity in vitro on human bronchial muscles have shown that the bronchorelaxing potency of terbutaline is about half (7/12) that of salbutamol (15). In the present study we have compared doses of 0.1 (= 100 μg) and 0.2 mg of salbutamol with 0.25 mg and 0.50 mg terbutaline, which gives a dose ratio of 0.6 rather than 0 5 Thus we gave a slightly larger relative dose of terbutaline.

Persson and Olsson (12) found isoprenaline to be 17 times more active than terbutaline on the isolated guinea-pig trachea, while Brittain (4) reports the same ratio to be 6 1 for salbutamol

Svedmyr and Thiringer (15) showed that i.v infusion of salbutamol raised the pulse rate only when FEV_1 had increased by about 1/3 of the maximum response. Inhalation of 0.2 mg salbutamol was found to induce very slight bradycardia (−1.3 to −3.8 beats/min) but no significant change after 0.4 mg and there was no significant change in BP after these doses (10). Warrel et al. (17) reported an increased heart rate but small effects on cardiac output in 3 patients. After inhalations of 0.2 mg salbutamol the average pulse rate increased by 7 beats/min, in one case 1.5 mg increased the pulse rate by 17 beats/min. In 16 patients inhalation of 0.3 mg salbutamol did not significantly change the pulse rate at rest or during and after work (9) Chatterjee and Perry (5) gave 0.5% aqueous solution corresponding to 5 mg salbutamol to 10 patients, the greatest increase in pulse rate being 10% from basal above normal resting values.

On the other hand terbutaline given as i.v injection of 0.2 mg, subcutaneous injection of 0.5 mg or orally (2.5–5 mg) also increases heart rate, cardiac output and stroke volume (12).

In the present study the control pulse rates, like those in most published studies, were not truly resting values (mean 81–83 beats/min) and the doses were not large enough to cause any objective circulatory effect or changes of ECG Therefore we cannot show any difference between the two tested drugs at true resting pulse rates or after larger doses. We can say however that after inhalation of doses which give around 50% increase of PEFR there were no objective side-effects from the heart, and pulse rate and BP seemed to return towards true resting values.

The duration of bronchodilation after inhalations of 0 2 mg salbutamol is reported to be at least 3 hours (6, 13). In the present study significant bronchodilation was still found 4 hours after 0 3 mg salbutamol and 5 hours after 0.75 mg terbutaline from 3 hours onward the effect of terbutaline was significantly bette As both terbutaline and salbutamol are considered to be unaffected by catechol-O-methyl transferase, this may be due to differences in regard to activity of other enzymes or binding in the tissue (4 12) The duration of bronchodilation after inhalation of 0.50 mg terbutaline was shown by Formgren (7) to be at least 5 hours; the time course was close to that seen in Fig. 1 in the present study The bronchodilatory effect of a single puff of terbutaline or salbutamol within 30 min was

clearly lower in our study compared to an added double dose (Figs. 1 and 2).

Arner (1) reports an absolute increase of PEFR of 30–40 l/min 5 hours after subcutaneous injection of 0.5 mg terbutaline in a material with a mean control PEFR of about 230 l/min and a minimum drug increase of 15%. Our material had a somewhat larger mean PEFR and reversibility and the total inhaled dose was 0.75 mg terbutaline. The mean increase of PEFR after 5 hours was then 70 l/min. When 0.50 mg terbutaline was given subcutaneously the effect on PEFR was somewhat enhanced compared to half that dose, but the pulse rate then increased.

After 7.5 mg terbutaline per os Formgren (8) reported an optimum effect after 3 hours and a duration of at least 7 hours; at this dosage the heart rate increased, some felt palpitations and 25% had tremor.

The present and earlier studies suggest that the degree and duration of bronchodilation with β-2-stimulating drugs are often greater after inhalation of the same dose than after parenteral therapy and side-effects are absent or smaller. Inhalation of these drugs, therefore, seems to be the safest and, in the ordinary patient, the most effective mode of administration. The risk of damage due to aerosol propellants should be [negl]igible, as no more that eight inhalations [(]4) should be needed and allowed in 4 hours.

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OBSERVATIONS ON TEMPORAL ARTERITIS

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Abstract. Clinical and laboratory findings in 53 consecutive cases of temporal arteritis, 48 verified at biopsy are reported. The remaining 5 had typical history and tender thickening of one or both temporal arteries. Signs of ocular involvement were present in 14 cases. The ESR was over 50 mm/h in all cases, in 42 over 100. Most cases had slight anemia, hypoalbuminemia and raised α-globulin and fibrinogen. Twenty-nine of 48 cases had elevated serum alkaline phosphatases. BSP tests (20 mg/kg b.w.) were carried out in 16 patients. All gave abnormal results. The test was more abnormal in the patients with ocular involvement. With this single exception there was no constant difference in clinical or laboratory findings between the groups with and without ocular engagement.

It is nowadays generally agreed that the only reliable therapy in temporal arteritis is long-term treatment with corticosteroids. This type of therapy however is not without complications, especially in elderly patients. One might thus hesitate to institute corticosteroid treatment in these patients if the diagnosis is not well established. In the typical case of temporal arteritis there is usually no doubt of the diagnosis, and treatment should be instituted promptly considering the risk of ocular involvement. There is, however, a large group of patients with the more unspecific picture of polymyalgia rheumatica. Many of these, but not all, develop a typical arteritis later, some of them with ocular complications. These patients should, of course, also have treatment as early as possible. There is, however, a considerable difficulty in finding these individuals in the large group of elderly patients with the fairly uncharacteristic picture of myalgic pains.

The aim of the present study has been to find out whether there are any clinical or biochemical parameters that might be of special value in the early diagnosis of temporal arteritis.

MATERIAL

The diagnosis of temporal arteritis was made in all patients with histological or clinical evidence of arteritis in the temporal arteries. Thus the finding of giant cells was not necessary for the diagnosis. Typical giant cells were seen in 41 cases. In 7 cases there were no giant cells, but intimal thickening, fibrinoid necroses and cellular infiltration in the media. Five patients refused biopsy. All had a typical clinical history, tender thickening of the temporal arteries on one or both sides, and showed an excellent response to corticosteroid treatment, for which reason they were included in the series. Our diagnostic criteria correspond to those recommended by Paulley and Hughes (22).

From 1954 to 1970 19 men and 34 women who fulfilled the criteria were recognized and treated in the Medical Department and the Department of Infectious Diseases. During this period we saw at least 18 more patients with convincing signs and symptoms of polymyalgia rheumatica. They all had similar biochemical findings and reacted promptly on steroid therapy. Since no obvious signs of arteritis were seen in the arterial biopsy specimens, these cases were not included in the present material. In another two cases a transient tumor swelling was noticed in the temporal area. Both had an accelerated ESR and a history suggestive of arteritis. Biopsies from both temporal arteries showed, however, normal histology in one of these cases, in the other no biopsy was performed. These cases were not included.

OBSERVATIONS

In Tables I and II clinical and laboratory data are given for the total material divided into two groups with reference to the existence of ocular complications.

Ocular manifestations

Two patients became bilaterally amaurotic. In one of them (case 43) the process spread to her second eye within the first 24 hours of treatment with corticosteroids in high doses. The other patient (case 46) had her second eye involved a year after the first attack while on reduced steroid dosage. In 8 patients only one eye was severely engaged with permanent total or almost total amaurosis (cases 4, 11, 15, 18, 26). One patient (case 19) developed a permanent visual field defect in one eye. I

Table I *Findings in 9 cases of temporal arteritis with ocular involvement*

Case no.	Sex	Age (y.)	Head ache	Myalgia	Fever	Hb	ESR	Total protein	Albumin	Globulins α-1	α-2	β	γ	Fibrinogen
4	♂	80	+	−	+	11.7	130	7.6	2.9	0.5	1.2	1.3	1.7	—
11	♂	71	+	−	+	11.0	132	8.4	2.4	0.6	1.2	1.2	3.0	—
15	♀	65	−	+	+	10.0	104	7.3	2.6	0.4	1.3	1.0	2.0	0.9
18	♀	71	+	−	+	10.0	1[illegible]4	6.3	2.0	0.5	1.1	1.0	1.7	0.8
19	♂	70	+	−	+	10.7	129	6.1	2.2	0.5	1.0	1.0	1.4	0.6
23	♀	73	+	−	+	10.0	91	6.9	2.9	0.3	1.0	0.9	1.8	0.7
26	♂	72	−	+	+	11.0	120	7.0	3.4	0.3	0.7	0.8	1.8	
27	♀	65	−	+	+	8.7	128	8.3	3.2	0.5	1.2	1.4	2.0	0.7
28	♂	71	+	+	+	9.0	100	6.8	2.7	0.5	1.1	1.2	1.3	—
33	♀	50	+	+	+	11.4	136	7.[illegible]	2.4	0.6	1.4	1.1	1.7	0.7
35	♀	81	+	+	+	7.8	145	7.8	3.0	0.6	1.1	0.8	1.6	—
43	♂	68	+	+	+	11.9	105							
45	♀	71	+	(+)	+	11.2	102	7.4						
46	♀	77	+	+	+	14.5	?							

Table II *Findings in 23 cases of temporal arteritis without signs of ocular involvement*

Case no	Sex	Age (y.)	Head-ache	Myalgia	Fever	Hb	ESR	Total protein	Albumin	Globulins α-1	α-2	β	γ	Fibrinogen
1	♂	69	+	−	+	11.5	102	6.4	2.1	0.4	1.1	1.2	1.5	—
2	♀	69	+	+	+	11.0	119	7.3	2.5	0.5	1.2	1.1	2.0	—
3	♀	68	+	−	+	10.1	125	7.2	2.6	0.5	1.4	1.1	1.6	—
5	♀	74	+	+	+	11.7	82	7.8	4.0	0.2	1.0	1.0	1.6	—
6	♀	74	−	+	+	9.6	112	7.3	3.0	0.4	0.7	1.0	2.2	—
7	♀	83	−	+	+	10.7	91	6.3	2.7	0.4	0.8	0.8	1.6	—
	♀	71	−	−	+	9.1	134	7.2	2.2	0.4	1.4	1.4	1.8	—
	♂	65	−	−	−	11.9	106	8.0	3.0	0.7	1.1	1.0	2.2	—
	♀	75		−	−	11.3	128	6.5	1.9	0.6	1.5	1.0	1.5	—
)	♀	57	+	+	+	13.3	90	8.2	3.0	0.4	1.2	1.2	[illegible]4	0.8
	♀	70	+	+	+	10.7	123	6.8	2.1	0.4	1.0	1.2	2.1	—
14	♂	81	+	−	+	14.2	111	6.1	2.0	0.6	1.0	0.9	1.6	—
16	♀	69		+	+	10.0	135	6.6	2.3	0.6	1.1	1.0	1.6	1.1
17	♀	69	+	−	+	10.0	123	7.0	2.1	0.6	0.9	1.7	1.7	0.7
20	♂	65	+	−	+	12.5	123	7.6	2.4	0.5	1.1	1.1	2.5	1.0
21	♀	76	−	−	+	10.4	125	7.0	2.2	0.6	1.1	1.1	2.0	0.6
22	♀	68	+	−	+	11.0	86	7.2	—	—	—	—	—	0.5
24	♀	65	+	−	+	9.7	97	—	—	—	—	—	—	0.8
25	♀	76	+		+	11.8	131	7.9	3.3	0.6	1.0	1.3	1.7	1.1
29	♀	75	+		+	13.1	99	7.7	2.9	0.2	0.6	1.1	2.9	0.7
30	♀	70	+		+	10.3	123	—	—	—	—	—	—	0.9
31	♂	73	+		+	10.5	125	7.3	2.3	0.5	1.1	1.5	1.9	0.7
32	♀	64	−	−	−	11.6	157	7.7	3.4	0.4	1.2	1.1	1.6	0.8
34	♂	63	+	+	+	13.0	81	6.1	2.8	0.3	0.8	0.3	1.3	0.6
36	♀	71	−	+	+	11.5	114	8.2	2.8	0.5	0.9	0.9	2.2	—
37	♀	79	+		+	12.4	59	7.1	3.4	0.4	0.7	0.7	1.2	0.6
38	♀	83	+	?	+	11.0	121	6.8	2.9	0.4	1.1	0.8	1.5	0.8
39	♀	71	+		+	13.9	141							0.8
40	♀	80	+	+		12.9	118	5.9	2.1	0.4	1.1	1.1	1.2	0.7
41	♀	63	+	+		10.3	111	8.5	2.7	0.6	1.3	1.1	2.8	—
42	♂	85	+	+	+	8.9	114	4.9	2.8	0.5	1.1	1.0	1.4	0.6
44	♂	62	(+)	+	+	11.0	116	6.7	3.3	↑	↑			—
47	♂	66	+	−	+	11.8	130	7.0	3.5		↑			—
48	♂	70	+	−	( )	14.8	62	6.8	2.9	0.3	1.1	0.8	1.7	—
49	♂	81	−	−		10.1	101	7.2	2.5	0.5	1.0	0.9	2.4	0.3
50	♀	70	−	+	+	11.1	119	7.3	3.2	↑	↑		↑	—
51	♀	73	+	+	+	14.0	105	6.7	2.8	↑	↑			
52	♂	69	−	−	+	10.4	130	7.1	3.2	↑	↑		↑	1.4
53	♂	75	+	−	+	10.7	112	7.8	3.0	0.4	1.1	1.3	2.0	—

Alkaline phosphatase	Total bilirubin	Thymol turbidity test	GOT	GPT	BSP	Latex fixation test
2.2	0.6	0.9	—	—	—	—
2.3	0.3		—	—	—	—
2.1	0.3	2.2	—	—	—	Neg.
4.4	0.3	0.2	19	16	120	—
2.6	0.2	0.2	36	51	115	Neg.
1.5 (7.5)	0.2	0.6	10	5	110	Neg.
3.1	0.4	2.1	—	—	—	Neg.
2.6	0.5	0.8	21	24	—	Pos.
5.4	0.3	1.7	20	23	90	Neg.
1.4	0.2	0.2	6	23	—	Neg.
3.6	0.2	0.6	85	179	—	Neg.

Alkaline phosphatase	Total bilirubin	Thymol turbidity test	*GOT*	*GPT*	*BSP*	Latex fixation test
Normal	0.3	0.1	—	—	—	—
Normal	0.1	1.2	—	—	—	—
3.6	0.3	0.2	—	—	—	(Neg.)
—	—	—	—	—	—	(Neg.)
6.6	0.3	0.2	—	—	—	—
—	—	—	—	—	—	—
4.6	—	—	—	—	—	—
3.4	—	—	—	—	—	—
8.8	0.2	0.4	—	—	—	—
1.0	0.2	0.5	—	37	—	Neg.
1.9	0.2	1.5	16	13	—	—
4.3	—	—	—	—	—	—
8.6	0.2	0.3	9	12	70	Neg.
2.3	0.2	0.1	12	18	100	Neg.
1.5	0.5	1.0	—	—	60	Neg.
1.7	0.1	0.3	9	5	40	Neg.
3.3	0.3	0.9	—	—	60	Neg.
2.0	0.3	0.2	8	7	—	—
3.1	0.2	0.7	—	—	75	Neg.
2.9	0.4	2.6	40	34	75	Pos.
4.0	0.1	0.2	—	—	—	Neg.
3.2	0.3	0.8	—	—	37	—
1.8	0.2	0.7	18	9	70	—
1.8	0.2	0.4	24	19	99	Neg.
1.5	0.2	0.8	—	—	—	Neg.
3.3	0.1	0.3	13	11	—	Neg.
4.1	0.6	0.4	21	27	70	Neg.
3.2	0.7	0.3	12	25	45	Neg.
4.1	0.7	0.1	69	33	—	Neg.
3.9	0.3	1.6	13	19	—	Neg.
1.6	0.3	0.6	21	12	—	Neg.
7.8	0.2	0.2	26	42	—	Neg.
2.8	0.2	0.2	36	72	—	Neg.
1.0	0.4	0.2	29	43	—	Neg.
2.3	0.2	0.7	21	26	—	Neg.
3.0	0.3	0.5	12	27	—	Neg.
3.0	0.5	0.2	8	12	—	Neg.
3.2	0.4	0.2	12	23	—	Neg.
2.1	0.3	0.2	28	70	—	Neg.

the remaining 3 cases there was no visual loss but fundoscopy revealed exudation, which in the absence of arterial hypertension and diabetes was regarded as due to the arteritic disease.

Age at onset

Our youngest patient was 50 years old at the onset of symptoms. The oldest was 85. Forty-seven of our 52 cases were 65 years or older.

Heredity

Cases 23 and 19 were sister and brother living in the same town but in separate households. Both patients had their first symptoms within three months.

Seasonal variation

When the material was arranged with respect to the time of the year at which the first symptoms appeared, the distribution did not show an increased incidence in the summer months, as has been suggested by Kinmont and McCallum (18).

Concomitant disease

Six patients had an overt diabetes mellitus when first seen, 3 others developed glycosuria during prednisone treatment. Moderate arterial hypertension was present in 4 cases. With these exceptions all patients had a systolic pressure of 190 mm or less and a diastolic pressure of less than 100 mm.

Twelve patients had a history suggestive of rheumatoid arthritis, 3 with more or less generalized joint engagement, 6 with deformities of the finger joints.

Initial symptoms

Headache alone or in combination with pains and stiffness in muscles of the back and neck was first symptom in 39 patients, myalgic pain without headache in 9. Three patients had registered no pains at all but were admitted for fever and elevated ESR. The time that elapsed between first symptom and admission to hospital varied between 2 weeks and 1 year. Ten patients had a history of an earlier episode 2–5 years ago, with what they regarded as similar symptoms. On that occasion one patient had the diagnosis of polymyalgia rheumatica at another hospital, two were diagnosed as "facial neuralgia" two as "facial paralysis" and one as "parotitis". No patient gave a history of typical migraine.

Involvement of large vessels

In one patient (case 8) the pulsations of her radial artery became weaker during her stay in the ward and before therapy was started. She also had a lower BP in that arm than in the other. Some large arteries were auscultated according to Hamrin et al. (11) in 20 patients. No significant murmurs were heard. Case 4 died during the first month of treatment from heart rupture. Autopsy revealed a disseminated arteritis involving the aorta, the coronary arteries and several branches of the carotid arteries but a normal histology in the femoral arteries. In another patient, who died from cardiac decompensation 6 months after therapy was started, there was athero-

mastoids, no definite signs of an arteritis in the coronaries, but widespread inflammatory exudates in the media of the aorta.

Headache

In Tables I and II all patients having pains in their head or face are registered as having headaches. In most patients it was the initial and predominating symptom. In 8 cases, however it was preceded by a period of myalgic pains not involving the head or neck. Seven patients definitely denied ever having had a headache. It is interesting that 3 of them belong to the group of patients with ocular manifestations.

Myalgia

Myalgic pains were present in more than half the material. They were localized in proximal muscle groups of the limbs or in the muscles of the back in most cases. Two patients suffered intense pains in the calves of their legs.

Fever

Fever or subfebrile temperature was observed in all patients but one varying between 37.6 and 40°C. T h patients gave a history of "influenza" or "cold" 1 3 months before admission. X-rays of the para-nasal sinuses showed mucosal swelling in 3 cases and an exudate also in one. Peculiarly enough, none of these 4 patients gave a history of a previous infection.

Hematologic findings

Hb was less than 12 g 100 ml in 43 patients with a minimum value of 7.8 (4). A moderate leucocytosis of more than 9 000 mm³ was present in 1 of 48 cases. ocytosis was not more common in the group with r involvement. Eosinophils were more than 400 in of 76 patients. Platelet varied within the range 200 000–500 000 in 37 cases, one patient had 550 000 mm³

ESR

In all patients the ESR (Westergren) was more than 50 mm/h, in all but 3 above 80. The ESR was not more accelerated in the group with ocular manifestations and it rapidly decreased on steroid treatment. The decrease closely paralleled the clinical improvement. An increase in ESR was usually the first laboratory sign of relapse when treatment was discontinued too early or when the steroid dosage was reduced too rapidly. Complete normalization of ESR during the initial period of treatment was no guarantee against relapse.

Plasma proteins

Serum electrophoresis was carried out in 46 patients. The albumin content was reduced in most patients with minimum value of 1.9 g 100 ml. α₂-globulin was more than 0.8 g/100 ml in 40 cases and β-globulin more than 1.1 in 11 cases. The haptoglobin value was over 200 mg 100 ml in all 26 cases examined, in of them it was more than 320 g/100 ml. γ-globulin was g 100 ml or more in 16 cases. The plasma fibrinogen in 26 patients examined was over 700 mg/100 ml in 13 and over 450 mg 100 ml in all patients.

Liver function studies

Serum alkaline phosphatase was determined according to Bessey Lowry and Brock in 48 cases and was over 25 U in 29. The values were normalized on treatment even in those cases who relapsed later. An elevated serum GPT value was found in 7 of 31 patients, an elevated GOT value in 2 of 30. The serum bilirubin levels and the thymol turbidity test were normal in all cases examined.

The bromsulphalein test (BSP) was made with 20 mg BSP/kg b.wt. as recommended by Castenfors and Hultman (7). (The series of BSP tests had to be discontinued because of complications observed in connection with the test in a number of patients suffering from other diseases.) T1/2 values for BSP in blood were determined from the elimination curves between 30 and 120 min after injection. The normal range for T1/2 in this hospital is 10–25 min. The BSP test was performed in 14 patients before any treatment was instituted and in another cases after few days of corticosteroid therapy. None of the patients had received morphine derivatives before the test. The test gave abnormal results in every case. Four of the five highest T1/2 values were found in the group with ocular involvement. The test was repeated once or twice during the first 9 months of treatment in 7 patients. The second and third T1/2 values were lower than the initial in all cases. The normalization or nonnormalization of the BSP T1/2 values did not predict the risk for relapse. The clinical development in relation to ESR, BSP and steroid therapy in 4 cases is shown diagrammatically in Fig. 1.

Liver biopsies

Percutaneous liver puncture was performed according to Menghini in 3 patients. In case 16 there was a moderate stasis in the fine bile ducts with intracellular deposition of bile pigment. This patient had normal cholecystography. In case 27 histology was practically normal with slight intracellular deposition of pigment but no bile stasis. Case 28 showed normal histology.

Various serologic tests on rheumatoid arthritis and LED were performed in 34 patients. The tests were strongly positive in case 28, who had no signs or symptoms of arthritis, and in case 29, who had no arthritic symptoms when tested, but developed arthritis 6 months later. All other patients had negative test.

Treatment

All patients were treated with corticosteroids, as a rule with prednisone. The initial dose was 60 to 80 mg d, in one case 15 mg d. This small dosage turned out to be insufficient for stopping the arteritis process which in a few days spread to a new area of the head. When the dose was raised to 60 mg d the progress stopped immediately.

In most cases corticosteroid treatment was effective on fever pains and ESR within few days. The dosage was subsequently reduced to 15–20 mg d and kept there for about a month. A further reduction was

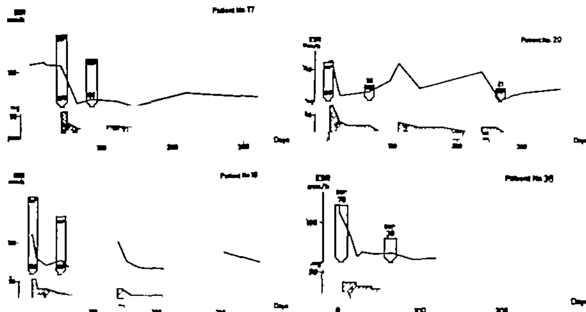


Fig 1 Variations in clinical symptoms, ESR, BSP with corticosteroid therapy in 4 typical cases. □ = corticosteroid dosage.

made under repeated ESR determinations to ascertain that there was no tendency to relapse. Three patients died during the first 3 months of therapy one from coronary thrombosis, one from thrombosis of the carotid arteries and one from pulmonary embolism. One patient had myocardial infarction after one month of treatment, but survived. In one subject bilateral biopsies of the temporal arteries were performed in the morning. At midday she received 20 mg prednisone and the same dose in the afternoon. When she awoke in the morning on the next day her second eye was amaurotic. It is difficult to tell whether this was purely coincidental or whether the catastrophe was provoked by the biopsies. Three patients developed glycosuria. Probably this should be regarded as more unmasking of an already existing diabetes.

One patient had reactivation of pulmonary tuberculosis and another moderate osteoporosis. Most female patients received nandrolone phenylpropionate (Deca-Durabol) as prophylactic against osteoporosis. W saw no case of female osteoporosis, although corticosteroid treatment in some cases as extended for more than 2 years.

DISCUSSION

It is very difficult to evaluate diagnostic criteria of temporal arteritis because it is impossible to find an acceptable "non-arteritic" control group for comparative studies. Since we cannot make sure that any elderly patient has not got an arteritis process somewhere in his body the only remaining possibility is to study patients with evident arteritis. If we can find a diagnostic pattern for cases of histologically erified arteritis, there are reasons to believe that the same pattern will also be applicable to cases in which the affected arteries cannot be identified or when they are not available for biopsy

Giant cell arteritis is almost exclusively reported in patients who are more than 50 years old. Some cases in younger persons have been reported, e.g. a histologically well documented case with temporal localization in a 35-year-old man (4) with a history of headache and weight loss but a normal ESR and serum protein pattern. Jankovics et al. (16) reported a 32 year-old man with disseminated giant cell arteritis This case had a normal ESR too. Cases younger than 50 years with typical histology and with the plasma protein changes usually found in arteritis of elderly patients, seem to be extremely rare.

Huges and Brownell (13) presented the opinion that granulomatous giant cell angitis of the central nervous system is a pathological entity with no correlation to temporal arteritis of the aged Giant cell granulomas are sometimes seen in the wall of the aorta and its branches in Ta-

kayashu's disease (2) and in giant cell myocarditis (21), two disorders with predilection for ages below 50.

It is interesting that polymyalgia rheumatica which nowadays is generally accepted as a manifestation of arteritis, is sometimes diagnosed at ages below 50 and sometimes with very moderate elevation of ESR (1, 6). Thus it is not impossible that in the future we shall have to accept another clinical appearance of temporal arteritis in younger ages. Still, there is little support for the opinion that temporal arteritis with risks of ocular involvement should be suspected in cases below 50 and with an ESR less than 40 mm.

In recent publications, especially from Scandinavia (10, 20) a low incidence of ocular complications is reported in polymyalgia rheumatica, even in series with a high frequency of positive arterial biopsies. In our material there were severe ocular complications in 10 of 53 patients, and a total incidence of ocular manifestation of more than 25%. Obviously visual loss is a stronger motive for asking medical advice than is headache and this group of patients is probably overrepresented in a medical department that gets arteritic patients from the eye clinic as well. Still, we want to stress that the risk of visual ...lications must not be underestimated (9, 12).

As can be seen from our material, it is not ...ible to predict from the localization of the ... which cases are going to have ocular complications. Temporal arteritis without headache was as common in the group with ocular involvement as in the rest. The existence or lack of pulsations in the temporal arteries is also of very limited prognostic value (17). The ESR elevation, anemia and leucocytosis are not more pronounced in the ocular involvement group. In our material the only finding that has been constantly different in the two groups is the BSP test, which was more abnormal in the group with ocular involvement.

The plasma protein pattern in our material corresponds very well with what is reported by several investigators: a constant elevation of α- and β-globulin concentrations with normal or moderately increased γ-globulins. It must be stressed that this type of pattern is not seldom seen in malignant diseases, especially hypernephroma, and we support Hamrin's (10) recommendation that urography ought to be performed in every case of suspected polymyalgia rheumatica or temporal arteritis. We have seen one man with severe myalgic pains in the calves and another with multiple necroses of his fingertips which initially were attributed to a disseminated arteritis. Both had a plasma protein pattern suggestive of polymyalgia rheumatica or temporal arteritis and both had a hypernephroma.

Although most investigators seem to accept temporal arteritis as a disseminated systemic process (15), very little is known of the effect of the disease on different organs. Renal failure is reported in a few cases (3). Impairment of the liver function in polymyalgia rheumatica was first reported by Klijn and Boomgaard in 1963 (19) who described three cases with pathological BSP test and increased alkaline phosphatases. Already in 1962, however, Boersma and Kerst (5) reported a correlation between the increase in alkaline phosphatases and BSP retention in "anarthritic rheumatoid arthritis". Terwindt and Knoben (23) studied a case of polymyalgia rheumatica with temporal arteritis (without giant cells). Alkaline phosphatases were increased to 35.5 King-Armstrong units with predominance for the liver isoenzymes. Also SGPT was increased. The BSP test was pathological. Light microscopical examination showed a normal histology. Electronmicroscopical examination revealed mitochondrial changes of unspecific nature of a nature seen in a number of different hepatopathies. After one month of prednisone treatment, serum enzyme concentration and BSP test were normalized.

In our material all cases investigated with BSP test had an abnormal T1/2. The BSP test was abnormal even in cases with normal alkaline phosphatases. Alkaline phosphatases were increased in more than half the material. SGOT and SGPT were rarely increased. It is interesting that alkaline phosphatase and BSP elimination test are the laboratory tests most sensitive to the inflammatory process in polymyalgia rheumatica and temporal arteritis. Both tests are sensitive to obstruction of the bile ducts (14) and a moderate bile stasis was also seen in the liver biopsy from one of our cases. The BSP test is reported to be pathological in most cases of rheumatoid arthritis (8). In a 32-year-old woman with Takayashu's disease and an ESR about 100 mm, a BSP test was almost normal.

Castenfors et al. (8) in their study of rheumatoid arthritis, found a close relationship between the BSP retention and the activity of the disease. The strongly pathological results of the BSP tests in our patients with ocular engagement might be interpreted as a sign of generalized dissemination of the process. During treatment the BSP values decreased, in most cases towards normal. There was no close correlation between the reduction of the BSP values and the clinical development of the disease.

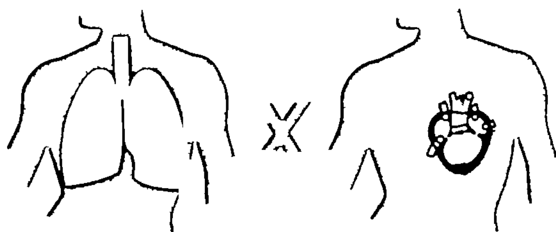
The characteristic combination of laboratory findings in temporal arteritis is not very specific. *Thus the laboratory findings will be of limited* value as an aid to the diagnosis of polymyalgia rheumatica in the absence of apparent signs of arterial engagement. When seen together with the usual signs of active systemic disease, ESR, α-2-globulin (haptoglobin) fibrinogen, an elevated alkaline phosphatase level will easily be misinterpreted as a sign of malignancy especially in the elderly patient. The rapid normalization of the laboratory findings on steroid treatment will strongly support the diagnosis of polymyalgia.

Considering the risk of severe side-effects from prolonged corticosteroid therapy in elderly subjects it would have been desirable to have some parameter telling which patient should have more intensive or prolonged treatment. The predictive value of all variables studied by us was low. The clinical course was essentially reflected by changes in the blood proteins. For long-term control purposes no biochemical factor studied gave better information than the ESR.

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CALCITONIN IN THE TREATMENT OF HYPERCALCAEMIC CRISIS

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Abstract. The hypocalcaemic effect of synthetic porcine calcitonin in hypercalcaemic crisis has been studied in 49-year-old man suffering from hypernephroma with skeletal metastases and 10-year-old boy with primary hyperparathyroidism. In both patients calcitonin induced prompt reduction of serum calcium concentration and pronounced clinical amelioration. This made possible successful removal of parathyroid adenoma in the young boy. No undesirable side-effects were observed during treatment with calcitonin.

Hypercalcaemic crisis is a serious, often fatal condition. Common symptoms are muscular weakness, vomiting, dehydration, constipation, and various mental disturbances, e.g. confusion. Pronounced hypercalcaemia may very rapidly lead to coma and death from cardiac arrhythmia or renal failure (2, 9, 18, 28, 30, 35). Hypercalcaemic crisis may be seen in various disorders of calcium metabolism. Most often it seems to appear in primary hyperparathyroidism or malignant disease (30). Several agents are able to lower the serum calcium concentration. Most of these are not efficacious in a hypercalcaemic crisis, however, since they act too slowly or may be nephrotoxic.

Calcitonin is known to lower serum calcium concentration in various hypercalcaemic conditions. The present paper reports on the effect of calcitonin in two patients suffering from such severe hypercalcaemia that it may be termed hypercalcaemic crisis.

MATERIAL

Patient 1 was 49-year-old police officer ho had complained of backache and tiredness for few months. He gradually developed polydipsia, polyuria, and anorexia. He suddenly went into confusion, and was taken to hospital. When first seen by us he was psychotic, confused as to time and place, excited, and at times aggressive. Physical examination showed nothing abnormal, except for a varicocele of the left side.

Laboratory examinations revealed Hb 10.3 g/100 ml, ESR 87 mm/h, PBI 3.6 μg/100 ml, serum creatinine 3.5 mg/100 ml, and creatinine clearance 61 ml/min. Serum concentrations of sodium, potassium, chloride, and bicarbonate ere normal. Serum calcium as 7.9 mEq/l, serum phosphate 5.1 mg/100 ml, and alkaline phosphatases were 10 Bodansky units. Paper electrophoresis of serum showed slight decrease of albumin and moderate increase in α-2-globulin concentration. Bone marrow examination was normal. Urinary calcium excretion could not be measured before therapy was started. Renal angiography revealed tumour in the left kidney and several metastases were seen on X-ray of bones and lungs. Therefore there was strong evidence that the hypercalcaemia was caused by renal tumour with skeletal metastases.

During the first two days after admission the patient received infusions of saline, 3–4 l/24 h, and injections of glucocorticoids. Serum calcium ranged between 7.9 and 7.4 mEq/l (Fig. 1). On the third day he as given calcitonin with rapid improvement (see Results). Meanwhile we arrived at the probable diagnosis of hypernephroma with widespread metastases. Since surgical treatment as not considered possible, the treatment with calcitonin as stopped. I.v. infusions of sodium sulphate (3.89% solution) were given during continued glucocorticoid therapy. The variations in serum calcium concentration are shown in Fig. 1 and the variations in urinary calcium in Fig. 2. After few days the patient developed paraplegia due to crush fracture of thoracic vertebra. He died 21 days after admission.

Autopsy showed hypernephroma in the left kidney growing into the renal vein, the renal pelvis, and the adipose renal capsule. There were metastases in the peritoneum, the paraaortic lymph nodes, the lungs, the pericardium, the adrenal glands, and the lungs, and also disseminated skeletal metastases.

Patient 2 as 10-year-old boy ho had suffered from headache, abdominal pain, vomiting, increased thirst, weight loss, and tiredness for one year. He had been examined at the hospital of his home town several times. Serum calcium had not been measured. The symptoms ere regarded as psychogenic. Because of intense vomiting

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ATRIOVENTRICULAR BLOCK IN AORTIC INSUFFICIENCY

Mechanism ECG Features and Clinical Consequences

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Abstract A review of 250 patients with pure aortic insufficiency (AI) and 400 with second or third degree atrioventricular block (AV block) revealed eight patients, seven male and one female, who had both these conditions. The mean age of the eight patients was 51 years (range 39–60). The duration of cardiac symptoms varied from 0 to 30 years. The aetiology of the heart disease was rheumatic fever in four patients, luetic carditis in one, and unknown in three patients. Seven patients had second and third degree AV block and one patient had paroxysmal ventricular standstill. The escape rhythm in the seven patients was nodal, so it seems likely that the occurrence of AV block in patients with AI may be due to mechanical strain on the upper parts of the conduction system, secondary to left ventricular hypertrophy and dilatation. In five patients the onset of AV block was accompanied by severe left heart failure, including pulmonary oedema. The material illustrates that occurrence of AV block may be a contributory cause of sudden deterioration in otherwise well compensated patients with aortic insufficiency

Atrioventricular block (AV block) of second and third degree is an uncommon occurrence in pure aortic insufficiency (AI). However as the combination of the two conditions has several interesting clinical and theoretical features, it has been thought worthwhile to report eight cases of AI complicated by AV block.

MATERIAL

In 250 patients with pure AI, seen from 1941 to 1970, eight developed second and third degree AV block (3.2%).

In 400 patients with second or third degree AV block, considered for treatment with artificial cardiac pacemaker six had pure AI (1.5%).

Particulars of the eight patients are given in Tables I and II.

The criteria for the diagnosis of AI were a diastolic murmur in the second right intercostal space, together with peripheral pulse phenomena. In patients 3 4, 5, 6 and 7 the diagnoses were confirmed by thoracal aortography and by the presence of typical arterial pressure curves. In no patient could a systolic gradient over the aortic valve be demonstrated.

The ECG diagnoses were made according to the criteria of Goldman (5).

DISCUSSION

In the present material the frequency of AV block in AI is 3.2% and the frequency of AI in AV block 1.5%

Rasmussen (14) reporting 84 cases of AI, and Segal et al. (19) reporting 100 cases of AI, found no patients with second or third degree AV block. Leonard and Smith (9) found 3 cases of total heart block in a material of 342 patients with luetic AI and the combination has been reported in rheumatoid carditis (11) Thus, seen from the viewpoint of the AI, the combination is quite uncommon.

In several large series of patients with total heart block (2, 6, 8 12, 18 21) about 10% had valvular heart disease as the possible underlying cause of the block. Often the valvular disease is unspecified, but pure AI seems very uncommon. Campbell (2) found one patient out of 64 and Penton et al. (12) four patients with aortic valve disease out of 251 with total heart block while Rowe and White (18) found only two patients with aortic valve disease out of 278 with total heart block.

Four of the present eight patients had a past history of rheumatic fever In one patient lues could be suspected, but WR was negative. The past history of the remaining three patients gave no clue to the aetiology of the heart disease (Table I)

Table I. *Data on the eight patients included in the study Symptoms and treatment prior to occurrence of block*

Case no.	Sex	Aetiology	Age at onset of cardiac signs or symptoms (y.)	Cardiac symptoms prior to block, functional class	Medical treatment before block	Age at onset of block (y)	Present age (y)
1	♂	Luetic?	51	Slight congestive heart failure Class II		Unknown	54 (dead)
2	♂	Unknown	ca. 35	Palpitations Class I	Chinidine, dose unknown	47	48 (dead)
3	♂	Unknown	56	Congestive heart failure, angina pectoris Class II	Digoxin, 0.25 mg Propranylyti chloridum, 20 mg 4	57	57 (dead)
4	♂	Rheumatic	37	No subjective symptoms Class I		39	43
5	♂	Unknown	52	Congestive heart failure, angina pectoris Class II	Furosemide, 40 mg 3 times a week	52	56 (dead)
6	♂	Rheumatic	62	Severe angina pectoris Class II		60	65
7	♂	Rheumatic	ca. 15	No subjective symptoms Class I		43	43
8	♀	Rheumatic	ca. 30	Slight congestive heart failure Class II	Digoxin, 0.25 mg	60	60

Theoretically in rheumatic AI, the following mechanisms could be responsible for the formation of the block.

The original carditis could damage the conduction system as well as the aortic valve.

The focal pathological processes, especially calcification, around the valve could gradually over a period of years, extend to the conduction system.

The wear and tear on the heart caused by the AI could involve the conduction system.

None of the present patients had active rheumatic fever and only two had calcium deposits in the heart (nos. 2 and 5). In patient 2 there was calcification only in the aortic cusps and none around the conduction system and the heart block was transient. In patient 5 the heart block was chronic, suggesting irreversible anatomical damage, presumably calcification in the bundle of His. Calcerous deposits in the aortic region with and without valvular involvement may lead to hemiblock, bundle branch block and AV block (6, 7 10 15 17 22) and histological studies have shown infiltration of fibrocalcerous material in the conduction system as first shown in Scandinavian literature by Petersen and Hall (13).

Left ventricular hypertrophy and dilatation from any cause may also lead to atrioventricular and interventricular conduction disturbances through mechanical overstretching of the conduction system (16). Furthermore experiences with cardiac catheterization have shown that even the lightest pressure or stretching of the conduction system may lead to transient conduction disturbances.

Regurgitation from an incompetent aortic valve may give rise to minimal prolonged trauma to the subaortic region, which in turn will produce scarring and endocardial thickening and even formation of aneurysmatic sacs into the septal myocardium (4) These lesions may well interfere with the function of the conduction system. Bis

Table II. *ECG and clinical findings at occurrence of block and effect of treatment*

Case no.	Symptoms at onset of block	ECG prior to block	Type of block	Characteristics of the escape rhythm	Chronic or transient block	Treatment and initial effect
1	Unknown	Unknow	Third degree AV block	Nodal (rate 40 beats/min, QRS showing left hypertrophy and strain)	Chronic	Folium digitalis (100 mg/d.), uncertain effect
2	Fatigue, dyspnoea	First degree AV block, left hypertrophy and strain	Third degree AV block	Nodal (unchanged QRS, rate 50 beats/min)	Transient	Digoxin and diuretic, some effect
3	Syncope, dizziness	Left hypertrophy and strain, incomplete LBBB	Paroxysmal ventricular asystole, 6–7 sec	No escape rhythm observed	Transient	Digoxin and diuretics, the patient refused pacemaker No more syncopes
4	Pulmonary oedema	Left hypertrophy and strain, incomplete LBBB	Third degree AV block, alternating with second degree, Mobitz II 2. 1 and 3 1 AV block	Nodal (unchanged QRS, rate 50 beats/min)	Transient, later chronic	Pacemaker immediate effect on cardiac incompensation
5	Congestive heart failure, angina pectoris	No previous ECG	Third degree AV block	Nodal (unchanged QRS, rate 45 beats/min)	Chronic	Pacemaker no effect on cardiac incompensation
6	Congestive heart failure	N previous ECG	Probably third degree AV block	Atrial fibrillation, bradycardia (rate 40 beats/min, QRS showing RBBB)	Chronic	Pacemaker gradual decrease of symptoms
7	Pulmonary oedema	Left hypertrophy and strain	Second degree AV block, Mobitz II 2. 1 alternating with third degree AV block	Nodal (unchanged QRS, rate 40 beats/min)	Transient	Pacemaker no symptoms after few hours
8	Pulmonary oedema	Left hypertrophy and strain	Second degree AV block, Mobitz II 2. 1, alternating with third degree AV block	Nodal, (unchanged QRS, rate 50 beats/min)	Transient	Pacemaker good effect on cardiac incompensation

(1) reports the case histories of five patients with AV block, supposedly due to jetstream lesions from mild senile aortic incompetence.

Complete AV dissociation was observed in all our cases. In patients 2, 4 7 and 8 the escape rhythm was nodal, i.e. the heart rate was about 45–50 beats/min and the QRS pattern was identical to that seen during sinus rhythm, indicating total AV block. In patient 6, who had atrial fibrillation, the ventricular rate was regular 40 beats/min, indicating nodal rhythm. As seen from Table II, the ECG of patient 1 is almost identical to those of patients 2 and 7 during third degree AV block. Patient 2 had first degree AV block and short episodes of second degree (2 1) before and after occurrence of ventricular standstill lasting 6–7 sec.

These ECG findings localize the lesions in the conduction system to the bundle of His or the AV node, while the impulse propagation in the distal parts of the conduction system is intact (3 17). The changing and passing nature of the block

might indicate that the damage in the conduction system is of a more functional than anatomical nature.

As appears from Table II, the block was seen simultaneously with an episode of acute left heart failure in five patients. In two of them there was a rapid, but gradual deterioration in the months prior to the discovery of the block. In one patient the block was discovered in connection with fainting episodes, but there was no heart failure.

In patients with normal myocardium, sudden bradycardia will lead to a decrease in cardiac output of a very short duration In a few minutes the heart rate and diastolic filling will find a new state of balance and normal cardiac output will be restored. However in patients with latent heart failure there is a critical heart rate at which the heart performs best, if the heart rate increases or decreases, the cardiac output is reduced (20) In AI the heart works with an increased diastolic filling and the capability for compensating bradycardia by increasing the diastolic filling is diminished. Therefore patients with AI of many years duration, on the verge of heart failure will stand the added strain of sudden occurrence of AV block very badly even with so slight bradycardia as seen in the present tients. The occurrence of block will lead to id development of left heart failure perhaps en pulmonary oedema.

The patients became at least initially asymptomatic when treated with pacemaker Thus it would seem that the block was a primary factor in producing pulmonary oedema.

Sudden death occurs in about 5% of relatively well compensated patients with pure AI. The causes are thought to be ventricular fibrillation rupture of the aortic cusps or inversion of the aortic valve following bacterial endocarditis (19) The present observations point to the fact that sudden deterioration in otherwise well compensated patients with AI may be due to occurrence of second or third degree AV block.

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STATISTICAL EVALUATION OF LIVER SCANNING IN COMBINATION WITH LIVER FUNCTION TESTS

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Abstract The diagnostic information of liver scanning has been evaluated statistically A focal defect in liver scan can be considered highly reliable abnormal finding. A diffuse decrease in liver radioactivity is of limited value, because such finding is often obtained not only in patients suffering from diffuse liver disease but also in patients ith small focal liver lesions or with no liver disease. By statistical comparison between liver scanning and number of liver function tests the diagnostic value of liver scanning was found to be superior to any of the liver function tests studied. This was true for liver diseases classified both as focal and diffuse. However, in focal liver diseases serum alkaline phosphatase was abnormal almost as often as liver scanning, and the combined use of this laboratory test and liver scanning seems to be very reliable diagnostic procedure in detecting focal liver lesions, especially metastases in the liver Multiple discriminant analysis clearly demonstrated the diagnostic value of performing liver scanning in addition to liver function tests for separating patients with normal liver from those with focal liver disorders. Liver scanning was also of some value in distinguishing patients with normal liver from patients with diseases generally considered as affecting the liver diffusely

Liver scanning has been performed with several different radioisotopes. Initially radioiodinated rose bengal was used (4) but, owing to its fast biliary excretion, liver scans obtained with this substance were sometimes difficult to interpret. Liver scanning has since been done mostly with colloidal ^{198}Au (16) ^{99m}Tc (7) or ^{113m}In (6). The value of liver scanning, especially in detecting localized liver diseases, has been well documented (11). Some comparative studies on liver scanning versus liver function tests have been carried out (5 8, 12, 14 15) However no statistical evaluation of the liver scanning information in discriminating between different types of liver diseases with the combined use of liver function tests has been published.

The aim of the present in estigation was to compare the diagnostic value of liver scanning and a number of liver function tests in different types of liver disease and to analyse the diagnostic power of combining scanning and function tests in discriminating focal and diffuse liver diseases.

MATERIAL AND METHODS

Patients

Liver scanning was performed in 405 patients with various diseases. The diagnosis of liver disease in 237 patients as verified histologically in 199 cases by liver needle-biopsy biopsy taken at laparoscopy or operation, or by autopsy In 38 cases with final diagnosis of liver disease the diagnosis was made by clinical and laboratory examination. Histological verification was obtained in 96 cases considered as having normal liver and in the remaining 72 cases this conclusion was reached by clinical and laboratory data.

Liver scanning

The equipment used was the Picker Magnascanner III. ^{198}Au gold colloid was injected so that the radioactivity dose was 250 μCi. Liver scanning was started 30 min after the injection. The liver was scanned first in the anterior and then in the lateral projection. The scans were primarily classified as either normal or abnormal. Liver size—normal or increased—was evaluated independently of this classification. The abnormal scans were divided into three subgroups depending upon hether the decrease in radioactivity was considered focal (single or multiple defect), diffuse or diffuse-and-focal. The scan diagnosis here called diffuse as used hen the radioactivity over the right liver lobe in the anterior projection was less than 6 000 cpm and no distinct focal defect could be recognized.

The evaluation of the liver scans was made by the two authors with no knowledge of the patient hen

Table I. *Liver scan diagnoses in patients with normal livers and various liver diseases*

Verified diagnosis	Total	Scan diagnosis: Normal	Focal	Diffuse	Diffuse and focal	Increased liver size in scan	%
Normal liver	168	133	2	31	3	53	32
Metastatic liver	93	7	39	23	24	75	81
Biliary disease	49	29	6	11	3	20	41
Portal cirrhosis	25	5	2	13	5	21	84
Hepatitis	14	7	1	6		7	50
Fatty degeneration	13	4		8	1	9	69
Hepatoma	12	1	6		5	11	91
Polycystic degeneration	8	1	5	2		5	62
Cardiac cirrhosis	7	1		5	1	6	86
Lymphoma	6			5	1	5	83
Liver necrosis	4		2	1	1	2	
Liver rupture	4		4			2	
Subphrenic abscess	1			1		1	
Echinococcus cyst	1		1			1	

reading the scan. For statistical analysis patients with liver metastases, hepatoma, polycystic liver degeneration, traumatic liver rupture and echinococcus cyst were considered as having focal liver disease. Portal and cardiac cirrhosis, acute and chronic hepatitis and fatty degeneration of the liver were regarded as diffuse liver diseases. Other diagnoses were omitted in this classification.

Liver function tests

The liver function tests studied were the serum total bilirubin, glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), lactate dehydrogenase and alkaline phosphatase (AP). Standard analytical methods were used.

Normal range of values: Bilirubin 0.1–1.2 mg/100 ml (equal to 2–20 μmol/l), GOT < 20 U/l (30°C), GPT < 20 U/l (30°C), LDH < 180 U/l (30°C), and AP 13–48 U/l (37°C).

Statistical evaluation

Multiple discriminant analysis was performed with a computer to assess the diagnostic usefulness of liver scanning. This procedure is a multivariate method, the prerequisite for which in its present form is that the final diagnoses are correct. The first task in the discriminant analysis was to find the linear combinations of variables, which in this study were a number of laboratory parameters and liver scan diagnoses expressed numerically in order to allow computer processing. The linear combinations of variables—expressed in the figures on the Y space, i.e. the discriminant space—give a better image of the difference between the diagnostic groups than the original variables. Even if the diagnostic groups that are compared do not seem to present any statistical difference with respect to a certain variable, a significant separation may be found because of the linear correlation of two or more variables. Multiple discriminant analysis provides an answer to the three main questions: a) Is there a significant difference between various groups when several variables are taken into account? b) What is the reason for the significant difference between tested groups? c) To which group does a certain patient belong on the basis of measured multiple variables?

Multiple discriminant analysis is particularly useful in analysing more than two groups, in which case regression analysis cannot be used. The results of the multiple discriminant analysis in this paper are demonstrated geometrically in a 2-dimensional system, which shows the degree of separation between the groups tested. The main purpose of this statistical method in the present study was to evaluate the diagnostic method of liver scanning in combination with some laboratory tests. For further details on this statistical procedure the reader is referred to Cooley and Lohnes (2) and Lasted (10).

The statistical analysis was performed at the Computing Centre, University of Helsinki, using an IBM 1620 computer. The non-parametric χ^2-test used for evaluating differences in frequency and the regression analysis for calculating the correlation between various laboratory parameters were performed with the computer's library programmes.

RESULTS

The patient material is presented in Table I. Liver scans from 168 patients with a non-liver disease were regarded as abnormal in 21% of the cases and in 32% of these patients the liver was enlarged. In 7.5% liver scanning was normal, though liver metastases were demonstrated histologically. Biliary disease resulted in abnormal scans in 41 of the cases, mostly diffuse scans. In portal cirrhosis the most common finding was a diffuse scan and increased liver size. Half the pa-

Table II. *Results of various laboratory tests performed in patients with different liver scan diagnoses*

	Scan diagnosis											
	Normal			Focal			Diffuse			Diffuse and focal		
Variable	Mean	S.D		Mean	S.D.		Mean	S D		Mean	S.D	
Bilirubin (mg/100 ml)	0.99	0.21	124	3.41	0.80	53	1.25	0 20	84	4.76	0.95	32
GOT (U/l, 30°C)	23.0	2.4	160	44.2	5.0	55	41.5	9.5	97	49 7	5.0	40
GPT (U/l, 30°C)	33.7	5.6	124	47.0	4.1	41	57.3	13 6	79	128	54	35
LDH (U/l, 30°C)	209	10	97	604	94	31	243	18	65	775	21	19
AP (U/l, 37°C)	61 7	7.2	157	140	13	59	65.1	4 9	98	126	11	40

tients with hepatitis had a normal scan, while the most frequent result in fatty liver degeneration was a diffuse scan. Of the 12 cases with primary liver malignancy only one was missed by scanning. In polycystic liver degeneration the scan was mostly focal. The major finding in cardiac and portal cirrhosis was a diffuse scan. Among the limited number of patients with lymphomas af fecting the liver (five cases of Hodgkin's disease and one lymphosarcoma) necrotizing liver disease of various etiology liver rupture and the single cases of subphrenic abscess and echinococcus cyst, all liver scans were classified as abnormal.

The results of the liver function tests obtained in the different types of liver scan diagnoses have been compiled in Table II. In patients with a focal or diffuse-and-focal scan the bilirubin values were considerably higher than in patients with a dif fuse scan. No significant differences were found in GOT values between the various diagnostic groups of pathological scans. Although the mean GPT value in diffuse-and-focal scans was higher than in the two other categories of abnormal scans, the difference was not very significant considering the large dispersion of the GPT values in the diffuse-and-focal group. High LDH values were typical of focal scans, while the differences between normal, diffuse and diffuse-and-focal scans were small. In patients with focal and diffuse-and-focal scans the mean AP values were about twice as high as in patients with normal or diffuse scans.

The diagnostic accuracy of liver scanning and liver function tests was compared statistically for focal and diffuse liver diseases. The statistical evaluation was performed non-parametrically be cause defining liver scans in terms of focal and diffuse decrease in radioactivity does not allow a parametric approach. For both focal and dif fuse liver diseases, scanning was superior to any laboratory test studied (Table III). However in focal liver diseases AP indicated liver disease with a diagnostic reliability almost equal to liver scanning.

The correlation between the parametric liver

Table III. *Abnormal liver scans and results of various laboratory tests in patients with normal liver or liver disease classified either as focal or diffuse*

	Normal liver			Focal liver disease				Diffuse liver disease			
		Total (n)	Wrong (%)		Total (n)	Right ( )	χ^2		Total ( )	Right (%)	χ^2
Abnormal scan	35	168	21	99	118	84	110 7*	42	59	71	49 4*
Increased size in scan	53	168	32	94	118	80	64.2	43	59	73	30.5
Bilirubin (>1.2 mg/100 ml)	4	105	4	21	89	24	16 8	14	49	29	19.8
GOT (>20 U/l, 30°C)	31	140	22	61	101	60	36.4	35	57	61	28 0
GPT (>20 U/l, 30°C)	32	104	31	46	80	58	13.2	32	51	63	14 4
LDH (>180 U/l, 30°C)	36	84	43	39	58	67	8.2	22	44	50	0 6
AP (>48 U/l, 37°C)	39	138	28	84	100	84	72 1	29	56	52	9.7

Values > 6.6 are considered statistically significant.

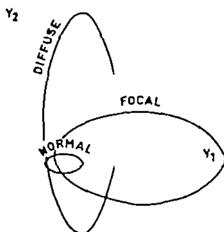


Fig 1 Geometrical demonstration of the results of multiple discriminant analysis in patients with normal liver and liver disease classified either focal or diffuse. Four discriminant functions (serum bilirubin, GOT LDH and AP) were analysed.

The ellipses define regions including 90% of the points of the group. A considerable overlap between the various diagnostic categories, especially patients with normal liver and those with diffuse liver disease, is apparent.

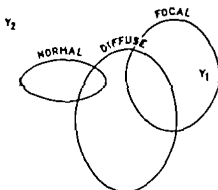


Fig. 2 Geometrical demonstration of the results of multiple discriminant analysis in patients with normal liver and liver disease classified as either focal or diffuse. Six discriminant functions (abnormal liver scan, increased size in scan, serum bilirubin, GOT LDH and AP) were analysed.

The ellipses define regions that include 90% of the points of the group. A high degree of separation between the various diagnostic categories, especially patients with normal liver and those with focal liver disease is demonstrated. The value of performing liver scanning in addition to liver function tests (see Fig. 1) is obvious.

function tests is given in Table IV In view of the fairly close correlation between GOT and GPT these tests were considered as giving approximately similar information in this study was therefore decided to omit one of these to reduce the complexity in performing the discriminant analysis. GPT was omitted because of the small number of values available in this study

Multiple discriminant analysis was first performed using the information obtained from the determinations of bilirubin GOT LDH and AP There was a very great overlap between patients suffering from a non-liver disease and patients with either focal of diffuse liver diseases (Fig. 1) When the information obtained from the liver scans—both abnormality and increase in liver size were taken into account—was included in the analytical procedure, a much better separation of the three diagnostic categories was achieved (Fig. 2) This is particularly true for discrimination between normal livers and focal liver diseases.

Table IV *Calculated correlation coefficients (-values) for various laboratory parameters in the patient material*

	Bilirubi	GOT	GPT	LDH
GOT	0.345			
GPT	0.122	0.473		
LDH	0 093	0.207	0 095	
AP	0.375	0.215	0 048	0.185

DISCUSSION

Since its advent, the main purpose of liver scanning has been the detection of space-occupying lesions of the liver and thus the differentiation between focal and diffuse liver disorders. This has been the aim regardless of the radioisotope or type of scanning equipment used. In the present study we have used the conventional technique of linear scintillation scanning and radioactive gold colloid. Although the use of ^{99m}Tc should be preferable to ^{198}Au in view of its better counting statistics, a comparative study by Cantor et al. (1) showed that only approximately 10% more hepatic lesions were detected with ^{99m}Tc sulfur colloid than with ^{198}Au gold colloid in a study on 48 patients with a linear scanner With ^{99m}Tc Rissanen and Patomäki (14) found that the use of a linear scanner and a gamma camera gave an equal percentage of positive find-

ings, but that the combined use of the two sets of equipment gave a higher percentage of positive findings. The value of the liver scan does not therefore seem to depend very much on whether ^{198}Au or ^{99m}Tc is used, or whether the scan is obtained by a linear scintillation scanner or a scintillation camera. The higher resolution obtained with ^{99m}Tc might even lead to a higher number of false positive interpretations. Thus the two main advantages of using ^{99m}Tc instead of ^{198}Au, speed and lower radiation dose, are obviously of no importance for the conclusions made in this study from the statistical comparison of liver scanning and liver function tests. The conclusions are therefore most probably also valid for liver scanning with ^{99m}Tc using the gamma camera.

Gollin et al. (5) showed that the overall accuracy of liver scanning was greater than for any single laboratory test. In their study AP was next in order of reliability. In another study AP also gave the best correlation with liver scanning when the scans were classified as normal and abnormal (14). These results are in accordance with our data from a considerably larger patient material, but only as concerns focal liver diseases. In diffuse liver diseases AP was of very little value for the differential diagnosis, and of the liver tests studied only LDH was of less value than AP. All the laboratory tests studied were diagnostically inferior to liver scanning in this group of liver diseases because of the high frequency of pathological enzyme values in many patients with a non-liver disease. Liver scanning therefore seems to be helpful in the diagnosis of diffuse liver diseases, too, and not solely for distinguishing between focal and diffuse liver diseases.

False positive scans were obtained in 21% of the patients with non-liver diseases. This seems surprising and points to the possibility that, in patients suffering from various other diseases, liver function might sometimes have been concomitantly disturbed even though no diagnosis of liver disease could be made. However in only 2.4% was a normal liver interpreted as having focal defects, a figure similar to that obtained by Gollin et al. (5) and Nagler et al. (13). The false positive scan was thus almost always due to a diffuse decrease in radioactivity. The reason for the reduced radioactivity in many patients with non-liver disease is hard to explain, but the comparatively equal frequencies of diffuse scans and enlarged livers indicate that this could be due to a dilution in the liver of the constant amount of radioactivity administered to all patients. The value of the diagnosis diffuse scan is limited but could possibly be improved by administering radioactivity in proportion to body weight or surface.

In 72% of focal liver diseases a correct diagnosis of focal scan was obtained. In 25 of the patients with proved metastatic liver a diffuse scan was found. In several of these cases the surface and sometimes also the interior of the liver was examined, and almost invariably the liver metastases had a diameter of less than 3 cm, which was evidently not sufficient to produce focal scan changes. False negative scans in the sense of classifying the scan as normal were obtained in 4.8% of the patients with a verified focal liver disease. In these patients the small size of the lesions, mostly metastases, was not even able to produce a diffuse scan.

In 54% of the patients with a diffuse liver disease the scan diagnosis was also diffuse. In 17% focal defects were observed in diffuse liver disease, mainly portal cirrhosis. Similar observations in portal cirrhosis have been reported by others (9, 15). There were 29% normal scans in diffuse liver disease and, therefore, it is quite obvious that a normal scan does not exclude diffuse liver disease with the same degree of confidence as focal liver disease.

The great value of combining liver scanning and liver function tests in obtaining the ultimate diagnosis of focal liver disease is readily shown by multiple discriminant analysis. It also shows that the process of diagnosing diffuse liver disease benefits by liver scanning, despite the vast number of false negative scans. Focal scans were rarely obtained in patients with no liver disease. In three of the four cases falsely diagnosed by scanning as having a focal liver disease this was shown to be due to compression from diseases in adjacent organs, a phenomenon that has also been described by Freeman et al. (3). This may also explain some of the focal scans in patients with biliary diseases, because the focal defects observed were often in such a position that they could have been produced by compression from an enlarged gall bladder. Focal scans were also seen in liver diseases generally regarded as affect

ing the liver diffusely. In spite of the relatively large number of diffuse scans in diffuse liver diseases the diagnostic value of the diffuse scans is doubtful because of the high rate of such scans in patients with no provable liver disease. As a diffuse scan can sometimes be due to focal liver disease with minor lesions below the resolution level of scintillation scanning, the diffuse scan should always inspire further examination of the liver, especially by morphological methods.

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IRON STORES IN NORMAL MEN AND MALE BLOOD DONORS

As Measured by Desferrioxamine and Quantitative Phlebotomy

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Abstract. Iron stores available for hemoglobin synthesis have been determined in 11 male healthy volunteers and in 14 male blood donor volunteers by means of quantitative phlebotomy. The mobilizable iron stores in normal men averaged 790 mg with range of 160–1 390 mg. The blood donors had significantly lower values with a mean of 110 mg and range of 0–230 mg. The desferrioxamine (DF)-induced urinary iron excretion was found to be closely related to the available iron stores. The DF test could separate groups with different size of the iron stores. In subjects with iron deficiency and anemia the DF-induced urinary iron excretion was significantly lower than that of group with depleted iron stores but no anemia. This indicates that DF derives fraction of its iron from other sources than the iron stores. During reduction of the iron stores by phlebotomy there was successive decrease in the urinary iron excretion induced by DF in most subjects studied. A diminution of the mean cell volume and Hb content of the red cells did not occur until the iron stores were almost depleted.

Storage iron may be estimated by several means. These include histochemical evaluation of tissue iron, chemical assay of tissue non-hemin iron and measurement by phlebotomy of iron available for hemoglobin synthesis. More indirect methods are n absorption measurements and determination the saturation of transferrin. Measurement of mobilizable iron stores is probably the most ct method at present because it measures all s and forms of storage iron and it readily per ts quantification (1). It is, however the most convenient method and has been limited to a v suitable volunteers (1 15 17 25).

During the last years the desferrioxamine (DF) t has been presented as a con enient and simple ethod in assessing the iron stores (1 11 20, 24) In most studies concerning the evaluation of e DF test the iron stores have been assessed by semiquantitative techniques (11 14 24) Balcerzak et al. (1) found, however a good correlation between DF-induced iron excretion and mobilizable iron stores in normal prison olunteers and subjects with hemochromatosis. Lundvall et al. (21) have recently reported a similar correlation between the DF test and mobilizable iron stores in subjects with porphyria cutanea tarda No studies ha e, however been made concerning the precision of the DF test in relation to mobilizable iron stores in normals.

The aim of the present investigation was to get further information of the magnitude of iron stores in normal men and in blood donors by means of vigorous phlebotomy and to test the significance and limitations of the DF test in assessing the iron stores.

MATERIAL

Group I consisted of 28 male healthy volunteers in whom DF test was performed. None had history of hemorrhage or blood donation. Eleven of these subjects subsequently underwent quantitative phlebotomy as described below. Most of them were clerks and students entering the hospital transfusion service for the first time.

Group II consisted of 32 male blood donors, who for four years or more had regularly donated 5–6 U of 420 ml blood/year. Most of them had no iron supplementation. The DF test was performed about 2 months after the last donation. From this group 14 blood donor volunteers underwent quantitative phlebotomy. The mean number of donations of this selected group was 47 with range of 20–86.

Group III consisted of 33 healthy males in hom depleted iron stores and iron deficiency anemia were induced by vigorous phlebotomy. Most of them were subjects of groups I and II.

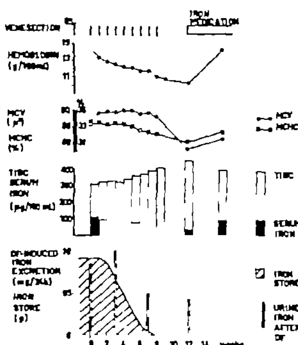


Fig. 1. Sequence of characteristic changes during development of iron deficiency anemia induced by quantitative phlebotomy. Each value is the mean of repeat observations performed in five healthy male volunteers with iron stores averaging 910 mg (range 810–1 050 mg).

METHODS

Storage iron readily available for Hb synthesis was measured by quantitative phlebotomy as described by Haskins et al. (15). Blood was removed by serial phlebotomies of about 450 ml. This included blood specimens taken at the end of each phlebotomy for determination of Hb, Hct, red cells, serum iron and total iron binding capacity (TIBC). The DF test was performed before, during and after phlebotomy at regular intervals. The design of the study is given in Fig. 1. The phlebotomies were repeated at weekly intervals until the Hb concentration 1 week after the last phlebotomy had fallen to approximately 10.5 g/100 ml or below this level. If during the next 3 weeks the Hb level was unchanged, and if the serum iron concentration and transferrin saturation were persistently low and the DF test indicated iron deficiency, it was concluded that the mobilizable iron stores were depleted. If the Hb concentration showed a significant rise, the phlebotomies were repeated until signs of iron depletion were achieved. About 3 weeks after the last phlebotomy a sternal marrow aspiration was done in the 11 normal subjects. The blood volume and total Hb mass were determined before and after phlebotomy in the group of normal males. In the group of blood donors this was performed only after the phlebotomies. Their blood volume at start of phlebotomy was considered to be 0.1 l greater than that determined afterwards. This value was derived from the average decrease in blood volume during phlebotomy found in 17 male volunteers (2).

Storage iron readily available for Hb formation was calculated as previously described (22).

All blood specimens were taken in the morning by venous puncture with the subject in recumbent position and in the fasting state. The Hb was determined photometrically as cyanmethemoglobin (6). A microhematocrit was spun in an International centrifuge. Red cells were determined with a Coulter counter. Serum iron was determined according to the method of Laurell as described by Weinfeld (27), and TIBC according to the method of Peters et al. (23). The smears from the sternal marrow were stained for hemosiderin according to the method of Hansen and Weinfeld (13).

Blood volume and total Hb mass were determined by the isotope dilution technique using radioactive chromium (26).

The DF test was performed as previously described (22). Ferrioxamine iron in the urine was determined in duplicate according to the method of Keberle (19) as modified by Lundvall and Weinfeld (20). The results are given in µg of 24-hour urinary iron/kg b.wt. and in mg/24 hours.

RESULTS

Mobilizable iron stores as determined by vigorous phlebotomy

Fig. 1 shows the experimental design and the characteristic changes following weekly phlebotomies in five young male volunteers without a history of blood loss. The first phlebotomy induced a fall in the Hb concentration. Then the Hb curve levelled off indicating an increased Hb synthesis which almost balanced the quantity of Hb removed each week. The amount of iron delivered to the erythroid marrow necessary to give this increased Hb production averaged approximately 150 mg/week. The transferrin saturation averaged 20% during this first stage of maximal iron mobilization. At the seventh week the DF test indicated iron deficiency, serum iron fell to values below 50 µg%, the TIBC saturation was less than 15% and the decrease in Hb concentration became more pronounced. When the transferrin saturation remained lower than 10% for 4 weeks, and the Hb level was unchanged in spite of discontinued venesections and the DF-induced iron excretion was low, it was considered that the available iron stores were depleted. This was confirmed by bone marrow examination which showed no stainable reticular iron and no sideroblasts. The subsequent iron medication gave a rapid increase of Hb.

The quantity of blood removed to bring the Hb concentration down to approximately 10.5 g/100 ml averaged 9 phlebotomies or 4.2 l blood in the

Table I. *Results of quantitative phlebotomy in 11 normal men and in 14 blood donors*

Subject no.	Age (y.)	Weight (kg)	Before (B) and after (A) phlebotomy	Hb (g %)	Hct (%)	MCV (μ^3)	MCHC (%)	Fe's (μg %)	TIBC (μg %)	TIBC satur (%)	DF-induced iron excretion in urine (mg/24 h)	DF-induced iron excretion in urine (μg/kg/24 h)	Mobilizable storage iron (mg)
Normal men													
7	27	65	B	14.0	42	93	33	100	336	30	0.82	12.6	1354
			A	11.4	34	85	34	23	509	4.5	0.30	4.6	
8	24	73	B	13.3	39	87	34	157	365	43	0.82	11.2	1052
			A	10.5	33	77	32	32	442	7.3	0.44	6.0	
9	33	72	B	14.4	44	89	33	72	308	23	0.66	9.2	992
			A	11.1	35	88	32	31	374	8.3	0.35	4.9	
10	23	80	B	13.7	41	87	33	105	320	33	0.89	11.1	885
			A	10.4	33	78	32	34	456	7.5	0.53	6.6	
11	23	69	B	14.9	46	85	32	76	330	23.0	0.90	13.0	821
			A	10.3	34	81	30	47	475	9.9	0.40	5.8	
12	24	82	B	14.3	43	86	33	166	259	64	1.00	12.2	812
			A	9.6	30	77	32	28	464	6.0	0.43	5.2	
13	21	75	B	16.5	47	87	35	108	275	39	0.78	10.4	667
			A	9.5	31	79	31	22	387	5.7	0.36	4.8	
14	20	68	B	14.4	42	95	34	163	362	45.0	0.78	11.5	664
			A	10.6	35	85	30	37	479	7.7	0.39	5.7	
15	22	59	B	13.5	40	85	34	121	342	35	0.80	13.6	614
			A	9.3	28	80	33	29	416	7.0	0.27	4.6	
16	25	80	B	14.3	45	87	32	162	301	54	0.78	9.8	293
			A	9.6	31	78	31	28	480	5.8	0.42	5.3	
17	25	61	B	14.2	41	87	35	121	296	41	0.54	8.9	177
			A	9.6	30	81	32	25	427	5.9	0.27	4.4	
Blood donors													
18	25	65	B	14.8	46	92	32	124	434	29	0.58	8.9	249
			A	10.4	33	85	32	23	408	5.6	0.31	4.8	
19	29	74	B	14.2	45		32	107	320	33	0.52	7.0	236
			A	9.5	31	84	31	30	406	7.4	0.35	4.7	
20	22	84	B	16.1	47	87	34	80	397	20	0.55	6.5	229
			A	10.7	32	80	33	43	471	9.1	0.40	4.8	
21	30	89	B	14.2	46	92	33	71	351	20	0.63	7.1	207
			A	10.8			33	20	427	4.7	0.42	4.7	
22	33	69	B	14.0	45		31	92	402	22	0.50	7.2	132
			A	10.8	34		32	26	451	5.8	0.35	5.1	
23	33	70	B	14.6	45	102	32	110	342	32	0.48	6.9	132
			A	9.6	31	100	31	38	427	8.9	0.33	4.7	
24	31	64	B	14.8	46	92	33	113	331	34	0.50	7.8	98
			A	10.5	33	86	32	32	537	6.0	0.33	5.2	
25	29	76	B	13.9	44		32	143	440	32	0.48	6.3	83
			A	9.6	32		30	34	530	6.4	0.36	4.7	
26	25	70	B	13.6	39	85	35	110	412	36	0.48	6.9	64
			A	10.2	32	82	32	54	435	12.4	0.36	5.1	
27	29	73	B	13.9	43	93	32	94	430	21	0.43	5.9	47
			A	9.8	31	82	32	22	403	5.5	0.34	4.7	
28	32	75	B	14.3	46		31	121	384	31	0.58	7.7	26
			A	9.3	31	86	30	35	464	7.5	0.40	5.3	
29	33	63	B	15.3	47	84	33	100	363	27	0.35	5.6	9
			A	9.9	31	77	32	30	403	7.4	0.31	4.9	
30	24	75	B	15.9	47	90	34	122	430	28	0.32	4.3	1
			A	10.5	32	91	33	25	472	5.3	0.36	4.8	
31	27	61	B	14.2	41	83	35	112	412	27	0.37	6.1	0
			A	9.3	29	76	32	22	464	4.7	0.21	3.4	

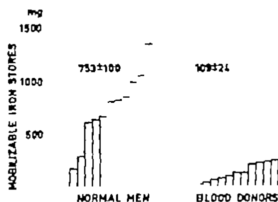


Fig 2. Mobilizable iron stores in 11 normal men and in 14 male blood donors.

group of normal men. The blood donors, however reached that value after 5 phlebotomies or 2.3 l removed blood.

The iron stores in the group of 11 normal males varied from 180 to 1 350 mg with a mean of 750 mg. The 14 blood donors had iron stores from 0 to 250 mg with a mean of 110 mg (Table 1 Fig. 2).

The bone marrows examined after phlebotomy showed no visible iron deposits in the 11 subjects studied.

DF-induced urinary iron excretion

(Fig. 3 Table II)

mean DF-induced urinary iron excretion in blood donors (0.46 ± 0.02 mg or 6.4 ± 0.2 μg/kg) was significantly lower than the mean value of 28 normal males (0.79 ± 0.04 mg or 10.5 ± 0.4 μg/kg) and significantly higher than the mean of 33 males with iron deficiency anemia (0.35 ± 0.01 or 4.9 ± 0.1 μg kg). However the degree of overlap between the DF test of the blood donors and the group of iron deficiency anemia was large. All normal subjects had values above the range of the iron deficiency anemia group. The DF-induced iron excretion in the 11 subjects studied by quantitative phlebotomy was 0.80 ± 0.12, which is of the same order as the mean for the whole group of normal men 0.79 ± 0.04 mg.

The relation between mobilizable iron stores and the DF-induced urinary iron excretion (Fig. 4)

The relation of DF-induced iron excretion to mobilizable iron stores was studied in 25 men, 11 normal males and 14 blood donors. A correlation coefficient of 0.83 ($p < 0.001$) was obtained. The equation of linear regression of DF-induced iron excretion (mg/24 h) on mobilizable iron stores (g) was $y = 0.40x + 0.47$. The residual standard deviation was 0.11 mg at a mean DF induced iron excretion value of 0.62 mg/24 h. The y-axis was intersected by the regression line at 0.47 mg/24 h. This predicted mean DF value of iron-depleted, non-anemic men was almost identical with that of 32 blood donors (0.46 ± 0.02) and was significantly higher ($p < 0.01$) than that of 33 male subjects with iron deficiency anemia and a Hb level of 10 g/100 ml (0.35 ± 0.01 mg/24 h, SD 0.06 mg) (Table II)

Serial determinations of the DF-induced urinary iron excretion showed a successive decrease during reduction of the iron stores by venesections in most subjects. However in 4 of the 11 normal subjects studied there was a slight increase of the iron excretion after DF during the first phase of phlebotomy (Fig. 1)

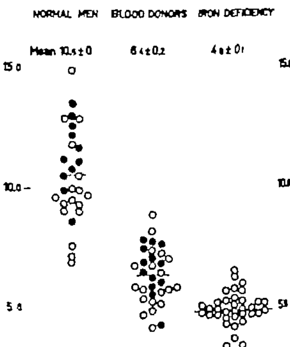


Fig 3 DF-induced urinary iron excretion (μg/kg b.wt/24 h) in normal males, blood donors and in iron deficiency induced by phlebotomy ● = values of subjects who subsequently underwent quantitative phlebotomy

Table II. *Age Hb level, serum iron, TIBC, TIBC saturation and DF-induced urinary iron excretion in the groups studied*

Means and S.E. of mean. The ranges are indicated within parentheses

		Age (y)	Hb (g/100 ml)	Fe/s (µg/100 ml)	TIBC (µg/100 ml)	TIBC satur. (%)	DF-induced urinary iron excretion (mg/24 h)	(µg/kg/24 h)
Normal men	28	26±1 1 (20–39)	14 7±0.1 (13.6–16.5)	132±7.9 (73–237)	326±7.3 (264–417)	41±2.7 (22–80)	0.79±0.04 (0.47 1.35)	10.5±0.4 (6 9–13.5)
Blood donors	32	32±0.7 (22 37)	14.6±0.2 (12.1 16.3)	101±4.7 (45–167)	379±9.2 (295–506)	27±1 4 (13–52)	0.46±0 02 (0.30–0 63)	6.4±0 2 (4.2–8.9)
Iron deficiency anemia	33	28±0.8 (20–35)	10.0±0.1 (8.2 11.4)	31±1.7 (19–61)	445±7.8 (360–537)	7 1±0.4 (4.5–13.1)	0.35±0.01 (0.21–0.53)	4.9±0.1 (3 4–6.3)

DISCUSSION

In the present study iron stores available for Hb formation in response to phlebotomy in 11 normal men showed a wide range of values (180–1 350 mg) with a mean of 750 mg. These results correspond well to those previously reported. Thus Hynes (17) found iron stores of 600 mg in one man by repeated phlebotomy An average of 1 200 mg was mobilized in two normal males by Haskins et al. (15) Prichard and Mason (25) mobilized 580 940 and 940 mg in three males. Balcerzak et al. (1) reported similar results in 11 normal prison volunteers with an average of 687 mg ranging from 130 to 1 900 mg.

Balcerzak t al however made no correction for iron absorption from food during the period of phlebotomy *In the present study an iron ab*sorption from food of 3 mg a day was assumed. This figure was based on the fact that male blood donors usually can donate 5 to 6 U blood per year without developing iron deficiency anemia This implies a retention of 3–4 mg iron/day which is in agreement with the value of 3 mg/day reported by Finch et al. (9)

The DF-induced iron excretion in the 11 normal subjects with iron stores quantitated by phlebotomy was of the same order as that of the whole group of 28 young, healthy males (Table II) Thus, it is reasonable to assume that the determined mean value of mobilizable iron stores of 750 mg is representative for young healthy males

The similarity in the results of storage iron from different parts of the world (the US and Sweden) is of interest Geographical differences of the iron stores ha e been suggested (3).

The iron situation in regular blood donors has *been described in several previous publications* where indirect methods have indicated almost depleted iron stores (7 8 10, 11 24 27). The results of the present study confirm this and show that the amount of reserve iron is ery low in this group with a mean of 110 mg and a range from 0 to 250 mg.

The results have shown that the DF test could separate groups with different size of the iron stores. This has been demonstrated earlier by Ploem et al. (24) and Hallberg and Hedenberg (11)

Fielding et al. (8) using the differentiated DF

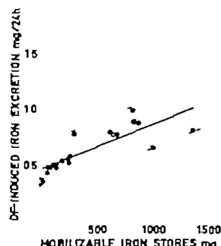


Fig 4 Relationship between mobilizable iron stores and DF-induced urinary iron excretion in 11 normal men and 14 blood donors. ● – values for the blood donors. The regression line and the 95% confidence limits are depicted. $y = 0.40x + 0.47$, $= 0.83$, $t = 7.3$, $p < 0.001$.

test, also found differences between such groups however the degree of overlap was very great. In the present study all normal subjects showed values above the range of the group of iron deficiency anemia. The degree of overlap was great between the blood donors and the group of iron deficiency anemia although the difference between the means was significant. This is in agreement with the results of chemical iron determinations in the bone marrow in similar groups of subjects reported by Weinfeld (27)

To evaluate the precision of the DF test, this test has to be related to iron stores more quantitatively determined. Hallberg et al. (12) found a high correlation between the DF test and the iron content of the liver determined chemically Balcerzak et al. (1) found a linear regression of DF-induced iron excretion on mobilizable iron stores determined in normal volunteers and subjects with iron overload. Lundvall et al. (21) have recently reported a good correlation between the DF test and available iron stores in subjects with porphyria cutanea tarda. In the present study there was also a high correlation between the DF induced urinary iron excretion and the iron stores determined by quantitative phlebotomy in normal males and blood donors. However for a given value of DF-induced iron excretion there was a ther wide range of the size of mobilizable iron res (Fig. 4) Predicted from the relationship of obilizable iron stores and DF-induced iron excretion in urine (Fig. 4) a mean DF value of 0.47 mg/24 h was obtained when the iron stores were zero. This finding indicates that DF derives a fraction of its iron from other sources than the iron stores, which is in agreement with several other reports (1 11 16, 18 24) The DF-induced iron excretion in the group of iron deficiency and anemia (0.35 ± 0.02 mg/24 h) was significantly lower than the mean value of the group mentioned above with depleted iron stores but no anemia. These results give support to the opinion of Hedenberg (16) and Karabus and Fielding (18) who suggested that DF chelates a fraction of its iron from the iron release at the heme breakdown in the reticuloendothelial (RE) cells. As this iron release is proportional to the iron content of the total Hb mass, a reduction of the latter should give less iron in the RE cell for DF chelation.

The sequence of characteristic changes during the development of iron deficiency anemia by quantitative phlebotomy corresponds well to those previously presented by Bothwell and Finch (3), Conrad and Crosby (5) and Weinfeld (27). When the serum iron level had fallen below 50 μg/100 ml and the saturation of transferrin to below 15% there was an impairment in the Hb synthesis. This led to the production of red cells with a decreased volume and a somewhat reduced Hb concentration. In most of the subjects there was a continuous decrease in the DF induced urinary iron excretion during the mobilization of the iron stores, which is in agreement with Balcerzak et al. (1) and Lundvall et al. (21) However in five of the 11 normal subjects (Fig. 2) there was an increase of the DF test at the initial phase of mobilization of storage iron (Fig. 1).

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BLOOD DYSCRASIAS ATTRIBUTED TO CHLORAMPHENICOL

A Review of 576 Published and Unpublished Cases

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Abstract. Reports of 576 published and unpublished cases of blood dyscrasias attributed to chloramphenicol have been studied. Pancytopenia (aplastic anaemia) was the commonest type of dyscrasia, accounting for about 70% of the cases; hypoplastic anaemia, agranulocytosis, thrombocytopenia and bone marrow inhibition made up the remainder. Among the patients with pancytopenia the outcome was apparently unrelated to the dose of chloramphenicol taken. However the longer the interval which elapsed between the last dose of chloramphenicol and the appearance of the first sign of the blood dyscrasia, the greater the mortality: nearly all patients in whom this interval was longer than two months died. The two sexes were approximately equally affected, and cases occurred at all ages. In most cases the condition for which chloramphenicol had been prescribed seemed not to justify its use.

Since 1950, when the first report appeared (16) many cases of aplastic anaemia and of related blood dyscrasias due to chloramphenicol have been described. Many authors (e.g. 2, 4 19 20) believe that the bone marrow is especially at risk in patients given chloramphenicol in high doses, for prolonged periods, or in repeated courses, and this view is expressed in the literature (5 10). There has also been an impression that women are affected relatively often (9 11). For example among 19 cases seen in the Netherlands, 14 were women (6, 15) but Sharp (17 18) has left this view in doubt. The prevailing views on the important factors in the production of marrow damage by chloramphenicol were inevitably derived from relatively small series of cases. Larger reviews have given insufficient details to allow more than rudimentary analysis. We have therefore undertaken a study of all the cases about which sufficient details were available, either in the original publication, or in private communications, in order to see whether any pattern could be discerned.

METHODS

W began by collecting the reports on adverse effects of chloramphenicol on the bone marrow cited in the Index Medicus for 1968 and earlier in Side Effects of Drugs, Vol. V (12) and earlier volumes, by Goodman and Gilman (5) and in the Extra Pharmacopoeia (10). We then added other papers cited in these reports and in current journals up to the end of 1970, and continued in this way to collect as many articles as we could find; the total number was 235 Since w were interested in case reports we excluded all the articles which lacked them, these were mainly reviews and warnings against the indiscriminate use of chloramphenicol. We also excluded reports of patients in whom the role of chloramphenicol therapy in the causation of their illness seemed uncertain Altogether we thus excluded 116 publications. From the remaining 119 papers we obtained 374 case reports. W also obtained details of 202 unpublished cases from private communications. Table I shows the sources of these cases. A check on sex, age, dosage and diagnosis was made to ensure that no case was included more than once. In fact only few instances of duplication were found.

Terminology

The terms used for the blood dyscrasias differed widely in the case reports. We have taken the term "pancytopenia to be synonymous with aplastic anaemia and preferably because all the elements of the bone marrow are depressed, not only the red cell series. The term "hypoplastic anaemia is here used for cases with non-progressive anaemia and hypoplastic rather than an aplastic marrow "Bone marrow inhibition we took to refer to lesser degrees of damage, for example maturation arrest.

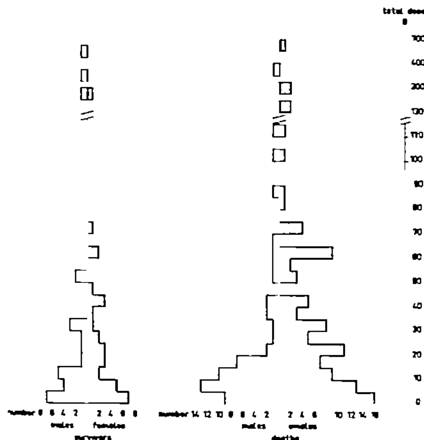


Fig. 1 Dosage of chloramphenicol in relation to outcome of pancytopenia in 204 cases.

Tabulations

For each reported case the following details were tabulated, if they were given:

1. The type of blood dyscrasia.
2. The dosage of chloramphenicol and the duration of treatment.
3. The latency i.e. the interval between the last dose of chloramphenicol and the appearance of the first symptom or sign of the dyscrasia, e.g. anaemia, spontaneous bleeding or infection.
4. Age and sex.
5. The indication for which chloramphenicol was prescribed.

From the tabulated data we attempted to assess:

1. The relative frequency of different blood dyscrasias.
2. Possible prognostic factors such as dosage and the latency period.
3. The age and sex distribution of cases, in relation to the age and sex incidence of the conditions for which the drug was prescribed.
4. In what proportion of cases the use of the drug appeared to be justified by the original diagnosis.

In assessing the age and sex distribution, the age and sex incidence of the conditions for which the drug was prescribed had to be considered. We therefore required data from a control population for comparison, and these were taken from a survey by Oliemans (14) of diagnostic groups occurring in five Dutch general practices. Unfortunately no data were available so far as age is concerned. We were especially interested in the frequency of respiratory infections, urinary infections, pertussis and typhoid fever. As typhoid fever is very rare in general practice, we also examined the in-patient statistics for the Netherlands for 1964 and 1965 (3).

In comparing our data with those from the control populations we assumed that doctors were equally likely to prescribe chloramphenicol for patients of either sex, and that men and women were similarly reliable in taking the drug. However differences in the sex incidence of various complaints could cause quite different prescription rates for chloramphenicol in the two sexes, and we therefore considered each diagnostic subgroup separately. As Berkson (1) has pointed out, lumping together heterogeneous groups can lead to wrong conclusions. At the time of our investigation typhoid fever could be considered as the only affection in which chloramphenicol seemed to be the drug of first choice.

χ^2-values were calculated to test the significance of comparisons between groups; where the numbers were very small a binomial test was applied.

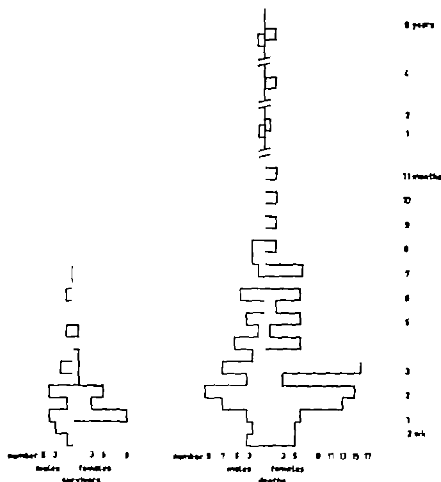


Fig 2 Latent interval between the last dose of chloramphenicol and the first manifestation of pancytopenia in relation to the outcome of the illness (249 cases)

RESULTS

Relative frequency of the various blood dyscrasias

Pancytopenia was by far the commonest type of disorder accounting for 70% of cases. The relationship of the latency period, dosage and age to the death rate was investigated first for this type of dyscrasia, since agranulocytosis and thrombocytopenia were much less frequent (18%). Hypoplastic anaemia and "bone marrow inhibition" which may represent milder forms of pancytopenia together accounted for 12%.

Dosage in relation to the outcome of the pancytopenia is plotted in Fig. 1. The proportion of deaths was approximately the same in patients who received a total dose of 20 g chloramphenicol or less, and those who received more. The same was true of the smaller groups of cases with agranulocytosis and thrombocytopenia. A total dose of 20 g is about the upper limit for an ordinary course of treatment with the drug. Data on the duration of treatment with chloramphenicol were not reported in sufficient cases to justify analysis.

Latency The interval between the last dose of chloramphenicol and the time of the first sign or symptom of pancytopenia in relation to the outcome of the illness is plotted in Fig. 2. Patients in whom the latency exceeded one year are shown separately in the Figure, since such a long interval raises some doubt about the connection with chloramphenicol. The median latency in the others was two months. It appears that survival is very rare in cases with a latency longer than two months but less unusual in those with a short latency (p 0.005) Cases with a latency of 7

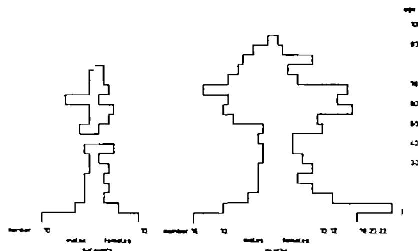


Fig. 3. Ages of patients with pancytopenia in relation to death or recovery (420 cases).

months or longer died. The same trend was apparent among the cases of agranulocytosis. The latency was reported in 13 cases, and the 2 in whom it exceeded 2 months both died. Of the remaining 11 with a shorter latency 9 survived. In all 5 cases of thrombocytopenia for whom the latency was stated, it was less than 2 months, and all 5 survived.

Table III. *Sex distribution (no. of fatal cases given within parentheses)*

Diagnosis	Males	Females	Total
Thrombocytopenia	12 (4)	9 (3)	21 (7)
Agranulocytosis	24 (16)	27 (13)	51 (29)
Pancytopenia (aplastic anaemia)	191 (134)	242 (182)	433 (316)
Hypoplastic anaemia	19 (0)	13 (0)	32 (0)
Bone marrow inhibition	15 (2)	24 (6)	39 (8)
Total			576 (360)

Table IV. *Indications for which chloramphenicol was prescribed*

	% of total
Respiratory tract infections	29
Urinary tract infections	14
Typhoid + paratyphoid fever	5
Acne	4
Pertussis	4
Prophylactically	4
Infections (unspecified)	10
Fever due to unknown cause	2
Unmentioned	28
	100

The ages of the patients with pancytopenia are plotted in Fig. 3. The proportion of survivors was similar in the different age groups and did not appear to differ for the two sexes. Survival among the patients with agranulocytosis and thrombocytopenia was also unrelated to age or sex. When the patients were divided into groups of those with respiratory diseases, urinary infections, typhoid fever and whooping cough, comparisons with the control group (14) showed no significant preference for either sex in the patients affected by chloramphenicol (Table II).

Sex and age incidence. Table III shows that none of the various blood dyscrasias showed a preference for one sex.

The indications for which chloramphenicol was prescribed are given in Table IV. Only in 5% of the patients was it used for typhoid or paratyphoid.

Other findings. The cases of pancytopenia included three pairs of twins, two uniovular and one biovular. Both members of each pair received chloramphenicol and developed pancytopenia (7, 13).

DISCUSSION

The findings indicate that pancytopenia (aplastic anaemia) is the commonest type of blood dyscrasia caused by chloramphenicol. The prognosis of chloramphenicol-induced pancytopenia appears

to be worse than for other types of dyscrasia caused by the drug and also to be similarly poor at all ages. It does not seem to be related to the total dose of chloramphenicol taken. It is, of course, another question whether the development of a blood dyscrasia is more likely in patients given high doses, as has been suggested in earlier studies (2, 4 19 20). The finding that a long interval between the use of chloramphenicol and the development of the pancytopenia is associated with about 100% mortality is puzzling. One possibility is that chloramphenicol produces two different types of pancytopenia which differ in latency and seriousness.

The long interval phenomenon might, however be an artefact. For example an intercurrent infection may be attributed to chloramphenicol only when it kills the patient. It is also conceivable that patients with a seemingly long latency have been ill for longer and that treatment of the dyscrasia is therefore begun relatively late so worsening the prognosis. Another possibility is that the retrospective diagnosis is altogether missed in survivors who develop transient pancytopenia after a long latency for the longer the interval the smaller the chance of obtaining a complete drug history from the patient. None of these or other less plausible explanations can be adequately tested on the data collected here.

Our investigation shows once again that adequately complete and reliable information about g treatment and adverse effects cannot be obtained retrospectively especially if they are reported by many different observers.

A prospective study would be capable of providing much more satisfactory data but since the incidence of blood dyscrasia is low of the order of 1 in 20 000 cases treated with chloramphenicol an enormous study would be needed which would be difficult to organize and sustain. One way of obtaining the records of all the patients receiving chloramphenicol in a given population would be to issue the drug only on a special prescription containing detailed information on diagnosis, age and sex in addition to the dose and the patient's name and address. This might well require legislation and the major effort of organization and communication involved would be expected to disturb the prescribing pattern for the drug. The data could perhaps be collected more satisfactorily in a drug monitoring system but no such system has yet been developed for adequate monitoring of drug use outside hospitals.

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A list of the 119 papers from which the 374 published case reports ere taken is available on request from L. Meyler Institute for Clinical Pharmacology Bloemsingel 1 Groningen, The Netherlands.

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COMPARISON BETWEEN ALPRENOLOL AND PROPRANOLOL AS ANTIHYPERTENSIVE AGENTS

A Double-blind Cross-over Study

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Abstract. A double-blind cross-over study comparing alprenolol and propranolol in equipotent β-blocking doses has been performed in 26 subjects with mild hypertension. Alprenolol and propranolol had an almost identical BP reducing effect. Propranolol reduced the heart rate significantly more than alprenolol, indicating different mechanisms of action for the antihypertensive effect. Subjective symptoms which could be interpreted as side-effects were few and transient with both drugs. Both alprenolol and propranolol seem well suited for the treatment of mild hypertension.

β-blocking drugs have been used in the treatment of angina pectoris, cardiac arrhythmias and hypertension for several years. Although the β-blocking drugs now in clinical use have different pharmacological properties, direct comparative studies on their clinical effects in long-term treatment seem to be lacking.

In a recent paper (2) a comparison was made between alprenolol and chlorthalidone as antihypertensive agents. It was found that alprenolol in a dosage of 100 mg 4 times daily reduced the BP significantly when compared to placebo. Chlorthalidone in a dosage of 50 mg a day reduced the BP significantly more than alprenolol given as 100 mg 4 times daily. When alprenolol was given in a higher dosage (200 mg 4 times daily) the reduction of the BP was greater and similar to that with chlorthalidone.

In the present study alprenolol and propranolol were compared as antihypertensive drugs when given in equipotent β-blocking doses individually titrated for each patient.

MATERIAL

The subjects were recruited from a material of 40 women previously participating in a trial with alprenolol and chlorthalidone (2). None of these women had a history or signs of cardiac or renal insufficiency. Twenty-seven of the women continuing to take alprenolol after completing the previous trial, and who were willing to participate, were given placebo for 6 weeks. Twenty-six women with systolic BP ≥ 150 and/or diastolic BP > 95 mmHg after this placebo period started a cross-over trial with alprenolol and propranolol. None of these women, aged 40–62 with mean age 53 years, had severe eyeground changes. Keith–Wagener–Barker grade I–II was found in 8 subjects, while no hypertensive changes were found in 18. All the 26 women completed the trial.

METHODS

Fig. 1 shows the design of the study. The periods with placebo were single-blind, while those with alprenolol and propranolol were double-blind, using a cross-over technique. The women were randomly allocated to alprenolol or propranolol as first active drug. The periods with placebo lasted for 6 weeks and the periods with alprenolol and propranolol each lasted for 8 weeks, the total time for the study including the placebo period being 22 weeks. All the participants started and completed the trial at about the same time.

BP was measured at the end of the placebo and treatment periods. A venous blood sample was drawn at the end of the treatment periods. BP was measured in the sitting, lying and standing positions after about 10 min rest. All the BP measurements were performed by the author using the same technique as in the trial with alprenolol and chlorthalidone (2). The laboratory methods were also the same. Electrolytes, bilirubin, SGOT, SGPT and uric acid were determined at the Central Laboratory, Sahlgrenska sjukhuset.

Earlier studies have shown that 40–50 mg propranolol induces about the same blockade as 100 mg alprenolol orally (1, 5). In the present study specially prepared tablets containing 150 mg alprenolol or 60 mg propranolol were given and considered to be equipotent doses. The tablets were administered 3 times daily. The women continued to take about the same dosage as before the placebo period. This dosage had been individually

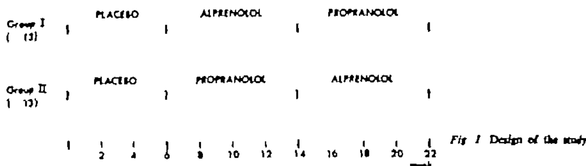


Fig 1 Design of the study

titrated for each subject. Fifteen women were given alprenolol 150 mg or propranolol 60 mg three times daily while 11 women, in whom the alprenolol dosage had been increased after completion of the previous trial, were given alprenolol 300 mg or propranolol 120 mg three times daily. After each treatment period the subjects returned the bottles, and the remaining tablets were counted.

The placebo tablets ere of the same appearance and taste as the alprenolol tablets they had previously taken, and no special information was given hen the placebo period was started. During the treatment periods the women were informed that they ere to receive two similar drugs in order to find out hich was the best for them.

Both active tablets ere identical in appearance and prepared to give the same dissolution rate as the corresponding commercially available tablets. When tested according to the method of US Parmacopeia XVIII, p. 934, using water at 37°C and 100 rpm, all the propranolol dose dissolved in less than 10 min and all alprenolol in less than 20 min. The tablets ere supplied by Hässle.

Statistical methods

Conventional statistical methods were used for the calculation of mean values and S.E. The significance of difference between sample means was estimated by Student's *t*-test for the means of differences between paired observations. In this way each subject acted as her own control throughout the study. Only subjects for hom observations were obtainable at corresponding times during the study were included. However except for weight, there were no missing data. The differences ere considered statistically significant for $p < 0.05$

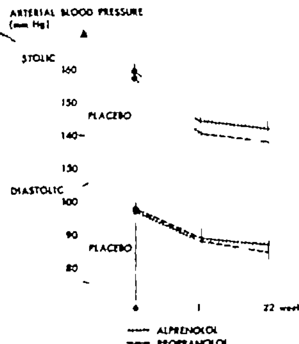


Fig 2 Means of systolic and diastolic BP in the sitting position after treatment with placebo, alprenolol and propranolol, respectively

RESULTS

Effects on arterial BP

The mean values for systolic and diastolic BP at the different determinations are given in Fig. 2. It will be seen that the initial BP values were about the same for the two groups. It can also be seen that the BP is lowest during the second treatment period irrespective of whether the patient received alprenolol or propranolol as the first drug. As may be seen from Table I the mean values found during treatment with alprenolol and propranolol were almost identical. The tendency was the same in all positions. Diastolic BP phase 5 is omitted in Table I as it closely followed phase 4.

Effects on heart rate

Propranolol reduced the heart rate by 7 beats/min more than alprenolol, as may be seen from Table I. This difference was statistically significant ($p < 0.001$). The range during alprenolol treatment was 54–84 during treatment with propranolol 50–72.

Effects on weight

It can also be seen from Table I that the mean weight was practically the same with alprenolol and propranolol.

Effects on laboratory data

As shown in Table I, serum uric acid tended to be slightly higher with propranolol than with alprenolol ($0.10 > p > 0.05$) No high individual values were recorded, none exceeding 5.5 mg/100 ml with alprenolol or 6.0 mg/100 ml with propranolol. Other laboratory data such as Hb bilirubin, SGOT and SGPT remained within normal levels. Two women with mild proteinuria before the study continued to lose the same amount of protein in urine during both treatment periods. One of these also had mild diabetes mellitus with glucosuria, which also remained unchanged.

Side-effects

All the women continued the medication during all the periods, although a few of them had to reduce the dosage during part of the time. At the beginning of the placebo period the medication with alprenolol was discontinued abruptly without the women noting subjective symptoms, except for one woman who complained of pulsations in her head. During the treatment periods the medication was started suddenly with a constant dosage during the whole period. One woman complained of headache and dizziness and had to reduce the dosage to half both during the alprenolol and the propranolol period. Her symptoms returned when she tried to increase the dosage. Another woman complained of weakness with alprenolol and had to halve the proposed alprenolol dosage. The symptoms remained the same when she started to take propranolol, but after two weeks treatment with propranolol she could take the dosage proposed from the beginning. A third woman complained of dizziness the first three days when starting to take alprenolol and had to reduce the dosage on these days, but could then take the proposed dosage without symptoms. It had been intended that these three women should receive alprenolol and propranolol in the higher dosage (300 mg and 120 mg, respectively three times daily) Another three women on the lower dosage complained of vomiting or dizziness during the first few days of active treatment, two of them when starting with alprenolol and one when starting with propranolol but they could take the originally proposed dosage without symptoms after the dosage had been reduced during the first two or three days.

Table I. *BP heart rate weight and serum uric acid after 8 weeks treatment with alprenolol and propranol, respectively*

		Alprenolol		Propranolol		Difference		Proba-bility
		Mean	S.E.	Mean	S.E.	Mean	S.E.	
Systolic BP (mmHg)								
Sitting	26	141 4	2.56	141.5	2.78	0.1	3 31	—
Lying	26	141.9	2.65	142.7	2.87	0.8	3.25	—
Standing	26	141.8	2.32	144.5	2.59	2.8	2.72	—
Diastolic BP (phase 4) (mmHg)								
Sitting	26	87.8	1.22	86 9	1 27	0 9	1.25	—
Lying	26	88.9	1 40	88.8	1.27	0.1	1 05	—
Standing	26	93.3	1.26	93 0	1.33	0 3	1 10	—
Heart rate (beats/min)								
	26	66.7	1.39	59 4	1.08	7.3	0 94	<0 001
Weight (kg)								
	25	72 2	3.15	72.1	3.22	0.1	0.21	—
Serum uric acid (mg/100 ml)								
	26	4 0	0.16	4 2	0 18	0.2	0.12	<0.10

Control of tablet intake

By counting the remaining tablets after each treatment period it was estimated that 84% of the alprenolol tablets and 88% of the propranolol tablets had been taken.

DISCUSSION

Alprenolol and propranolol reduced the systolic and diastolic BP of the subjects in the different positions to the same extent. Neither were great intra-individual differences observed when comparing alprenolol and propranolol.

The dosages given ha e been regarded as equipotent. Whether they are equipotent in fact may be a matter of controversy but they seem to be as equipotent as possible as indicated by previous investigators

When administered acutely by i.v. injections to hypertensive patients, alprenolol and propranolol ha e also been shown to reduce the arterial BP

to the same extent (4) In the study by Johnsson et al. (4) the hypotensive effect of propranolol was caused by a decrease of cardiac output. The calculated systemic peripheral resistance increased and this effect may partly have counteracted its antihypertensive action. After administration of alprenolol the cardiac output also decreased while the peripheral resistance was essentially unchanged (4) This difference between propranolol and alprenolol was assumed to be due to the intrinsic activity that alprenolol possesses in addition to its β-blocking effect (3)

The results in the present study seem to be in agreement with previous reports (6, 7) indicating that the differences between the acutely recorded hemodynamic effects of the two drugs also persist during long-term treatment. Thus the heart rate was reduced to a greater extent after propranolol than after alprenolol this finding possibly reflects a smaller cardiac output with propranolol than with alprenolol. As the reduction of the arterial BP was the same after the two drugs the vascular peripheral resistance might be lower during the alprenolol than during the propranolol period.

As noted in a previous paper (2) alprenolol unexpectedly raised serum uric acid moderately but significantly In the present study serum uric acid was raised to almost the same extent as during alprenolol treatment in the previous study Propranolol tended to raise the serum uric acid ore than alprenolol. The rise in serum uric acid probably not of clinical importance either for lprenolol or for propranolol.

Even quite high doses of alprenolol could be withdrawn suddenly in this trial and replaced by placebo without symptoms occurring. When starting again with alprenolol or propranolol in corresponding doses, relatively many patients experienced mild side-effects. Thus 6 out of .6 women had to reduce the dosage during the first few days although they had taken the same dosage without side-effects before the placebo period. It therefore seems advisable to increase the dosage successively when commencing treatment with alprenolol or propranolol.

The present investigation thus demonstrates that alprenolol and propranolol are equipotent antihypertensive drugs in patients with mild hypertension. As side-effects were few and slight, these agents seem well suited as antihypertensive drugs on this indication.

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RECURRENT THROMBOSIS IN A YOUNG WOMAN WITH A CIRCULATING ANTICOAGULANT DIRECTED AGAINST FACTORS XI AND XII

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Abstract. A young woman with discoid lupus erythematosus and potent circulating anticoagulant directed against factors XI and XII is described. The patient had no bleeding symptoms, but had had repeated episodes of venous thrombosis. The fibrinolytic activity of the vein walls was found to be decreased. It was apparent that circulating anticoagulant against factors XI and XII will not prevent thrombus formation in patient with low fibrinolytic activity of the vessel walls, hich is known to predispose to thrombosis.

Circulating anticoagulants may develop spontaneously even in the absence of primary haemophilia. Such anticoagulants ha e been demonstrated in patients with various diseases, such as tuberculosis, dermatitis herpetiformes, rheumatoid arthritis, lupus erythematosus, drug allergy and sometimes also in young and otherwise healthy women in the puerperium and in apparently healthy middle-aged and elderly persons (4 7 11 20, 21) The anticoagulants have been directed against AHF (factor VIII) and thromboplastin, and occasionally against factor IX, factor V and prothrombin (4 7 8 9 11 19 20 21) As far as we know anticoagulants against factors XI and XII have not been demonstrated in acquired diseases.

The young woman described here was found to have an anticoagulant directed against factors XI and XII. This patient had had repeated episodes of venous thrombosis and later developed discoid lupus erythematosus.

METHODS

Platelet and coagulation studies

Determinations of the platelet count, Duke and Ivy bleeding times, platelet adhesiveness according to Hellem's whole blood method and Salzman's method, coagulation time in glass and plastic tubes, recalcification time, one-stage prothrombin time, factor VIII, factor IX, prothrombin, factor VII and factor X (Owren P & P-test), fibrinogen and thrombin time were made by the proce dures described earlier (5 22, 23, 24). Platelet aggregation was studied at 37°C with platelet aggregometer obtained by G. V R. Born and in the way described by Cronberg (5).

Factors XI and XII ere determined by modifications of the methods described by Soulier and Prou-Wartelle (31) and Horowitz et al. (16). The test base used consisted of platelet-poor normal citrated plasma adsorbed with 30 mg celite/ml (Johns-Manville, Lompoc, Calif.) for 10 min to remove factors XI and XII. The powder was removed by centrifugation of the mixture twice, each time at 4 000 g for 20 min. The supernatant plasma was adjusted to pH 7.0 with 0.1 N HCl or 0.1 N NaOH and incubated at 37°C for 6 hours, during hich it was frequently shaken. The platelet count in the plasma to be tested was adjusted to 200 000/mm³ before freezing. The assay was performed in the following way: 0.2 ml of test base, 0.2 ml of Trombofax solution (Ortho) and 0.2 ml of the plasma to be tested in various dilutions were mixed and incubated for 6 min in new glass tubes in water bath at 37°C, after which 0.2 ml of 30 mM calcium chloride solution was added and the clotting time was measured. The activity of factor XI and that of factor XII were expressed relative to that found for normal standard consisting of pooled platelet-rich plasma (200 000 platelets/mm³) from 10 registered donors. *Factor XI* as assayed separately in some plasma samples using plasma from patient with severe congenital factor XI deficiency as test base in cephalin system. *Factor XII* was assayed separately in one-stage recalcification system using plasma from patient with Hageman trait (< 1% factor XII) as test base.

Test for circulating anticoagulant and normalization tests. The inhibiting effect of the patient's plasma on the recalcification time of normal plasma was determined. The highest dilution of the patient' plasma, capable of prolonging the recalcification time by 15 sec, was taken as the anticoagulant titre of the patient' plasma. Normalization tests ere carried out in the same way with the patient plasma as test base (26).



Fig. 1. (a) Occlusion of the proximal part of the left posterior tibial vein. (*b*) Occlusion of the right popliteal vein.

Two-stage method for assay of inhibitors against factor VIII and factor IX. One part of AHF concentrate or factor IX concentrate (containing about 300 units of AHF and factor IX, respectively) is incubated with (*a*) 3 parts of barbital buffer (pH 7.8, ionic strength 0.15), (*b*) 3 parts of normal plasma, (*c*) 3 parts of the patient's plasma in various dilutions for 2 hours at 37°C. Following incubation the blank and the mixtures of plasma and concentrates are assayed for residual factor VIII and factor IX activity according to the method of Nilsson et al. (23). The inhibitory activity of the plasma is expressed as the number of units of factor VIII or factor IX inactivated by 1 ml of the plasma.

Inhibitory activity against factor XI and factor XII. One part of normal plasma is incubated with (*a*) 1 part of barbital buffer, (*b*) 1 part of patient's plasma for 30 min at 37°C. After incubation the mixtures were assayed for residual factor XI and factor XII activity using celite-adsorbed plasma, congenital factor XI deficiency plasma and congenital factor XII deficiency plasma as test bases.

Fibrinolytic studies

The following determinations were made: fibrinolytic activity of plasma and resuspended euglobulin precipitate on fibrin plates, plasminogen (immunochemical method), inhibitors of plasminogen activation (urokinase inhibitors), α_2-macroglobulin (α_2M), antiplasmin and fibrin degradation products (FDP) (blood collected with EACA and thrombin). The methods have been described previously (13, 14, 25).

Venous stasis was induced by placing a sphygmomanometer cuff around each upper arm and inflating it to a pressure between the systolic and diastolic BP for 20 min. Blood samples for determination of the fibrinolytic activity of resuspended euglobulin precipitate were collected from each arm before induction of stasis and immediately before the cuff was deflated. The samples were drawn from an antecubital vein. The normal values range between 158 and 500 mm² (17, 30).

Fibrinolytic activity of the vein wall. A segment from a hand vein was excised under local anaesthesia (0.5% Carbocain). The veins were examined by Pandolfi's modification of Todd's fibrinolysis autography technique (17, 28). The activity was expressed in arbitrary units according to Pandolfi and Nilsson (28). Normal values for an arm vein are 6–11 arbitrary units (17).

CASE REPORT

On May 6, 1968, a 16-year-old female was admitted because of bilateral thrombosis in the legs.

Previously she had always felt well except for an increased bruisability of the legs for a short period in 1967. Mainly because of severe dysmenorrhoea the patient had been using oral contraceptives (Conlunett®) since March 1968. She was on the 14th day of the pill schedule.

On May 4, 1968, while watching TV, the patient experienced a sudden pain in the right calf, which swelled. On the following day the same symptoms appeared in the left leg. The patient consulted a practitioner who immediately referred her to the University Hospital in Uppsala.

Examination on admission (May 1968) revealed typical signs of bilateral thrombosis of the lower legs. *Laboratory studies and X-ray studies.* Hb 11.5 g/100 ml; WBC 8 000/μl with a shift to the left. ESR 91 mm/1 h. Fibrinogen 0.67 g/100 ml. Phlebography showed a 17 cm occlusion of the proximal part of the left posterior tibial vein (Fig. 1 *a*) and occlusion of the popliteal vein, from 10 cm below the knee joint to 3 cm above (Fig. 1 *b*). The patient was treated with heparin i.v., dicoumarol (AP®) orally and bed rest, and recovered without complications.

In 1969 the patient became pregnant. Her last menstrual period was on June 22, 1969. Pregnancy was uncomplicated until Dec. 28, 1969, when labour started and the patient had a small uterine haemorrhage. She was admitted to the Department of Gynaecology and Obstetrics.

Examination on admission (Dec. 1969) disclosed incomplete abortion of a 30 cm long macerated foetus. It was instrumentally removed. Pathological examination of the foetus revealed no gross malformation. The placenta was partially necrotic. The patient was sent home on Dec. 31, 1969.

Two days later she had a recurrence of the pain in the left foot and calf. She was again admitted to the hospital.

On admission (Jan. 1970) the patient had clear clinical signs of left lower leg thrombosis. This time, however, phlebography was not done. Body temperature 39.2°C, pulse 88, and BP 130/70. *Laboratory studies.* Hb 11.5 g/100 ml; WBC 5 000/μl, platelet count 156 000/μl. ESR

72 mm/1 h. Fibrinogen 0.5 g/100 ml. Liver function tests, including SGOT and SGPT revealed no abnormalities.

The patient was treated in the same way as before and recovered without complications. She was referred to the Coagulation Laboratory Malmö Allmänna Sjukhus, for further evaluation.

Examination revealed circulating anticoagulant. It was therefore decided to examine the patient again for any underlying disorder. In the meantime she had felt well.

At re-examination in Sept. 1970 she reported that she had had facial erythema in 1969 and somewhat more severe recurrence in the summer of 1970. A butterfly erythema covering both cheeks and the nose was seen (Fig. 2). Otherwise the physical examination revealed nothing remarkable.

Laboratory studies. The blood picture was normal. ESR 15 mm/1 hour. Liver function tests, including SGOT gave normal values. Serum cholesterol 146 mg/100 ml; serum triglycerides 64 mg/100 ml. Serum iron 147 µg/100 ml. Total serum protein 6.1 g/100 ml with slightly increased γ-globulin fraction of the polyclonal type. Immunoelectrophoretic analysis revealed IgG 1 570, IgA 180 and IgM 80 mg/100 ml; all within normal limits. No LE cells. Antinuclear antibodies were found in low titre, 1/10 (in May 1971 the titre was 1/100).

Coomb test was negative. No demonstrable antibodies against thyroid, kidney or parietal cells. Antistreptolysin and antistaphylolysin titres were negative. Tests for rheumatoid factor (Akryl) and anti-γ-globulin were also negative.

A dermatologist diagnosed the facial erythema as discoid erythematosus. A skin biopsy was compatible with the diagnosis.

At the last examination in Sept. 1971 the erythema had not disappeared, possibly because of her work as an assistant nurse with exposure to ultraviolet light. She otherwise felt well.



Fig. 2. Discoid lupus erythematosus with typical butterfly erythema.

RESULTS

Platelet and coagulation studies

Results of routine haemostatic tests are given in Table I. The patient had a normal platelet count and bleeding time and no signs of defective platelet adhesiveness or aggregation. Prolonged coagulation and recalcification times were constant findings. The one-stage prothrombin time

Table I. *Platelet and coagulation studies*

	3/70	6/70	12/70	Normal range
Bleeding time (min)				
Duke		2	2	1 5
Ivy		14	14	4–12
Coagulation time (min)				
Glass	28	23	25	6–14
Plastic	50	60	60	12 32
Platelet number (per mm³)	260 000	250 000	290 000	150 000–300 000
Platelet adhesiveness (%)				
Hellem whole blood	21	27		17–33
Salzman's method		20	19	20–60
Factor XI (%)		24	7	60–160
Recalcification time (sec)	340	330	450	120–160
One-stage prothrombin time (sec)	23[a]	20[a]	18[a]	13–15
P&P (%)	44[a]	44[a]	66[a]	80–120
Factor V (%)	75	100	92	80–120
Factor VIII (%)	60	82		60–160
Factor IX (%)		53	58	60–160
Factor XI+XII (%)	8	12	7	60–160
Factor XII (%)		5	5	60–160
Fibrinogen (g/100 ml)	0.27	0.31	0.33	0.20–0.40
Thrombin time (sec)	16	16	17	14–18
Anticoagulant titre	1 20	1 20	1 20	0
Platelet aggregation at 37°C with Born aggregometer				
ADP			Normal, second wave	
Adrenaline			Normal, second wave	
Collagen			Normal	

[a] Dicoumarol therapy



Fig 1 (a) Occlusion of the proximal part of the left posterior tibial vein. (b) Occlusion of the right popliteal vein.

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Inhibitory activity against factor XI and factor XII One part of normal plasma is incubated with ( ) 1 part of barbital buffer (b) 1 part of patient plasma for 30 min at 37°C. After incubation the mixtures were assayed for residual factor XI and factor XII activity using celite-adsorbed plasma, congenital factor XI deficiency plasma and congenital factor XII deficiency plasma as test bases.

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of the patient's serum revealed a mild anticoagulant activity of the fraction in the γ-region.

Fibrinolytic studies

The various components of the fibrinolytic system in the patient's blood were normal (Table III). On one occasion the amount of FDP in serum was increased. The response of the fibrinolytic activity to venous stasis of the arms was in the lower range of the normal values. The plasminogen activator content of the walls of superficial arm veins was subnormal.

DISCUSSION

The young woman presented here, who was admitted to hospital because of repeated thrombosis, was found to have a markedly prolonged coagulation time and low values for factors XI and XII, while other routine coagulation tests gave normal values. A circulating anticoagulant was demonstrated, and all tests performed indicated that this anticoagulant inhibited the activity of factors XI and XII. There was no evidence that the patient's anticoagulant was directed only against factor XI or only against factor XII. When the patient's plasma was added to normal plasma, it inactivated its content of both factor XI and factor XII, as assayed on test bases from patients with severe factor XI or factor XII deficiency. Nothing suggested that the patient had had a coagulation defect earlier. Josephson and Lisker (18) have described a case of congenital factor XI deficiency in which the condition was complicated by development of an anticoagulant. As far as we know no other reports are available on anticoagulants against factors XI and XII. As pointed out in the introduction, most acquired inhibitors of coagulation are directed against factor VIII, factor IX or thromboplastin. These inhibitors have been characterized as immunoglobulins (2, 8). In our case the inhibitor was recovered among the immunoglobulins, but the inhibitor was not characterized immunologically.

Acquired anticoagulants have been found mainly in disorders characterized by immunological phenomena such as collagen diseases, drug reactions, and in haemophiliacs following transfusions. In the patient described we do not know whether the discoid lupus erythematosus should be regarded as the cause of the anticoagulant. However in patients with discoid lupus there are often different immunological disorders without other signs of transition into the systemic form of the disease (1). With the exception of the positive ANF titre, there is so far no evidence of systemic changes in our patient.

Most patients with acquired anticoagulants have had a haemorrhagic diathesis as severe as that observed in the most severely affected haemophiliacs. Patients with factor XII deficiency have no increased bleeding tendency. Patients with severe or moderate factor XI deficiency have bleeding symptoms of roughly the same severity as those with mild haemophilia A or B. Our patient had no signs of an increased bleeding tendency but, remarkably enough, had had repeated episodes of venous thrombosis. It is known that factor XII deficiency offers no protection against thrombosis. Glueck and Roehll (10) and Hoak et al. (15) have reported two patients with factor XII deficiency and myocardial infarction and John Hageman died of pulmonary embolism (29). We have not found any reports of factor XI deficiency combined with thrombosis. But a few cases with circulating anticoagulants with thrombosis have been published. Bowie et al. (3) reported on 4 cases with thrombosis in systemic lupus erythematosus despite circulating anticoagulants. In these cases no phlebographic diagnosis of the thrombosis was made. Lechner (19) described a patient with an anticoagulant inhibiting the activation of prothrombin and with a history of thromboembolic episodes 4 years before the detection of the anticoagulant. Green and Rizza (12) have described a patient with myocardial infarction and a circulating anticoagulant against factor VIII. According to these authors the deficiency of a single clotting factor will not prevent thrombus formation in a patient with a predisposing vascular disease. It must be stressed that this refers to arterial thrombosis. The findings in our case show that factor XI and factor XII are not necessary for the development of venous thrombosis.

In our case the finding of such changes, probably capable of facilitating thrombosis, was noteworthy. In a study of 117 cases of idiopathic venous thrombosis of different parts of the body Isacson and Nilsson (17) recently showed that a high percentage (55%) of such patients had an abnormally low content of fibrinolytic activators

in the walls of the superficial veins. As many as 38 % of these patients had also a defective mobilization of endogenous fibrinolytic agents, as judged from an abnormally low fibrinolytic response to standardized stimuli, such as venous occlusion. In addition Åstedt et al. (33) have shown that the fibrinolytic activity of the vein walls is markedly decreased during pregnancy Åstedt (32) has also found that large doses of oestrogens (250 μg/d.) cause a decrease of the content of fibrinolytic activators in the vessels. The first attack of thrombosis in this patient appeared when she was taking P-pills, and the second during pregnancy Our investigations were performed 24 months or more after withdrawal of P-pills and 3 months or more after interruption of pregnancy We repeatedly found a slight decrease of fibrinolytic activators in the superficial vein walls and a slightly decreased release of activators during venous occlusion of the arms. During pregnancy and possibly also during the use of P-pills a further decrease of the fibrinolytic activity of the vein walls might be assumed. These changes in the fibrinolytic activity may very well have precipitated the thrombosis in our patient. It is quite clear that the circulating anticoagulant directed against factor XI and factor XII could not prevent thrombus formation in ; case.

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INTRACTABLE PAROXYSMAL TACHYCARDIA IN THYROTOXICOSIS SIMULATING MYOCARDIAL INFARCTION

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Abstract. The case of 59-year-old man, admitted to hospital because of precordial pain indicating acute myocardial infarction (AMI), is presented. ECG showed evidence of posterior wall infarction. The subsequent course was characterized by variety of cardiac arrhythmias, notably atrial fibrillation, treated with digoxin and for short time with β-blocking agent, and premature ventricular beats and short runs of ventricular tachycardia, treated with lidocain and procainamide. Transient bradycardia and A-V dissociation caused the patient to be transferred to coronary care unit to have cardiac catheter inserted. Four weeks after the acute episode intractable ventricular tachycardia caused the patient's death. At autopsy no evidence of AMI was found. Thyroxine levels in the plasma, reported post mortem, were in the hyperthyroid range. In retrospect evidence of masked thyrotoxicosis could be discerned. The occurrence of angina pectoris, atrial fibrillation and other more unusual cardiac arrhythmias in thyrotoxicosis are discussed. No previous report of thyroid hormone-induced fatal ventricular tachycardia appears to have been published. It is emphasized that the diagnosis of thyrotoxicosis should be considered in all less characteristic cases of cardiac arrhythmia, despite the concomitant presence of cardiac pain suggesting myocardial infarction.

The diagnosis and treatment of acute myocardial infarction (AMI) are increasingly being concentrated to specially equipped coronary care units (CCU) to which patients with precordial pain and/or arrhythmias are admitted directly or referred to from other hospitals. Inevitably patients in whom these symptoms on closer inspection turn out to represent diseases other than AMI will also increasingly be admitted to these specialised units.

The purpose of this communication is to focus attention on one differential diagnosis which it may sometimes be important to consider if treatment is to be successful.

CASE HISTORY

A 59-year-old man was in 1970 admitted to the CCU of Medical Department B, Rigshospitalet, with the diagnosis of tachyarrhythmia, secondary to AMI.

No family history of heart disease could be elicited. In 1959 the patient was discovered to have mild diabetes mellitus subsequently controlled by dietary restriction. In 1968 he was found to be moderately hypertensive with BP averaging 180/100 and eyeground changes corresponding to grade II retinopathy Standard ECG leads were normal at this time. His hypertension was treated with methyldopa (Aldomet®), 250 mg every other day During the same year he was admitted three times to physiotherapy department with the presumed diagnosis of spondylosis of the spine.

In Nov 1970 the patient for the first time experienced attacks of pain in the chin, radiating to the precordial area. At the same time he noted tendency to swelling of his ankles and slightly elevated body temperature (37 3–38.1°C) Following three consecutive attacks of rather severe pain in one day the patient was admitted to local hospital, where on the basis of the ECG provisional diagnosis of posterior wall myocardial infarction was made. Because of moderate signs of cardiac incompetence, the patient was treated with diuretics and digitalized. Four days after admission atrial fibrillation was noted, and the dose of digoxin was increased. As the atrial fibrillation proved hard to control, β-blocking agent (Aptin®) was added to the treatment on the ninth day

On following day episodes of bradycardia and syncope occurred. Digoxin treatment was accordingly discontinued, but the episodes of bradycardia continued. On the 12th day the patient was therefore transferred to this department to have cardiac pacemaker inserted.

When first seen at this department the patient was confused and sweating with regular pulse of 114 and BP of 170/80. Physical examination did not show cyanosis, signs of left heart failure or cardiac murmurs on auscultation The thyroid gland was thought to be of normal size. ECG showed regular sinus rhythm, Q wave in lead III of 0.4 mV and pronounced S–T waves in V to V Because of the recent episodes of bradycardia and syncope, an Elema pacing catheter was inserted into

the right ventricle and connected to a Medtronic pacemaker of the demand type.

Initially the patient was treated with diuretics, digoxin 0.25 mg × 2 daily, insulin 32 IU daily (Retard®) and glucocorticoids, started previously because of evidence of heart block. On the second day in this department premature ventricular beats were noted. Lidocaine treatment was accordingly started and continued for 2 days, during which time the incidence of ectopic beats was reduced to almost nil. Because of intermittent occurrence of atrial flutter, digoxin treatment in doses of 0.25 mg

daily was continued. 24 hours after discontinuation of lidocaine, periods with premature ventricular beats recurred, and procainamide in doses of 500 mg × 4 daily was now added to the regimen. This treatment appeared effective for several days, but from the 14th day of his stay in this department premature ventricular beats reappeared with increasing frequency and could not be suppressed by increasing the dose of procainamide to 750 mg × 5 daily. During the final night ventricular tachycardia occurred, developing into ventricular fibrillation, and proved intractable despite numerous attempts at defibrillation, the last of which for a short time re-established pacemaker rhythm without effective cardiac contractions.

Laboratory tests. The Hb content was slightly reduced throughout the patient's stay at the local hospital and in this department. ESR was initially normal and rose to maximally 55 mm/1 h. Serum concentrations of creatinine and electrolytes were consistently normal. Serial determinations of LDH activity in the serum showed normal values during the entire first hospitalization, while moderately elevated levels were found during the last 10 days in this hospital. There was no consistent trend in serial determinations during the latter period, the maximum being 525 U/l, minimum 370 U/l. Isoenzyme measurement showed the rise in LDH to be mainly due to increases in fractions 2 and 3, suggestive but not pathognomonic of myocardial damage. Serum cholesterol levels were repeatedly low normal or subnormal, with minimal value of 3.6 mmoles/l (normal values in this hospital 3.9–8.6 mmoles/l). Urinary excretion of VMA was normal. Fasting blood sugar values were abnormally high and there was a moderate degree of glucosuria throughout. Following the patient's death the serum content of thyroxin was reported to have been 241 nmoles/l (normal values 66–139 nmoles/l).

Autopsy report. The heart was enlarged, weighing 520 g. There was distinct left ventricular hypertrophy, the wall of the left ventricle measuring 17 mm. A careful search failed to show any changes in the myocardium compatible with infarction or fibrosis. The major coronary arterial vessels were of normal calibre with minimal arteriosclerotic changes, whereas the smaller vessels showed severe arteriosclerotic changes. The lungs were edematous with macroscopic changes indicating stasis and bronchopneumonia. There were signs of stasis in the liver and spleen. The thyroid gland was normal macroscopically.

DISCUSSION

The patient, when first admitted, presented with typical symptoms of AMI and ECG showed the changes characteristic of posterior wall infarction. In the course of the following four weeks until his death a number of arrhythmias occurred which were treated according to current principles, viz. atrial fibrillation with digoxin, A–V dissociation and bradycardia with a temporary pacecatheter, premature ventricular beats and episodes of ventricular tachycardia with lidocaine and procainamide. Despite these efforts death occurred due to intractable ventricular tachycardia and final pump failure. Nothing in this sequence of events was incompatible with the initial diagnosis of myocardial infarction, and yet the autopsy report failed to show any evidence of a recent infarction or myocardial fibrosis.

At the time of autopsy the result of thyroxin measurement in the serum became available and appeared to offer strong evidence that the patient had, in fact, had thyrotoxicosis. In retrospect, several features support the conclusion that the patient had hyperthyroidism and not an AMI. Both when first admitted to the local hospital and when first seen here, he appeared nervous, warm and sweated profusely. The pulse rate, apart from a few days during which he would appear to have developed A–V dissociation, was never below 80, and mostly above 100, even when regular. When comparing his weight with that registered during an admission in 1968 to a physiotherapy department, it is apparent that he had lost approximately 15 kg. The serum cholesterol levels were very low for a 59-year-old man with presumed coronary occlusion. Furthermore, although the ECG changes initially were compatible with a posterior wall infarction, these changes came and went with considerable rapidity also 3 weeks later, apparently to some extent depending on the pulse rate, indicating a transient myocardial ischemia. Finally, the LDH levels did not rise above normal until about 15 days following the acute initial episode, and then remained elevated to about the same level for the remaining days of the patient's life.

It seems reasonable to conclude that the course of this patient's illness was not due to an AMI, but to a masked and unrecognized thyrotoxicosis. Before accepting this conclusion it is necessary, however, to ascertain whether all of the features presented by this patient's case history can be reasonably ascribed to a state of hyperthyroidism.

Over the years the effect of thyroid hormones

on the heart has been the subject of much discussion and many reviews (2, 4 5 8 10 18 20, 21). Limiting the discussion to the present case, three questions may be asked.

1. What is the relationship between thyrotoxicosis and angina pectoris and ischemic heart disease?

2. What types of arrhythmias may be associated with thyrotoxicosis?

3 May arrhythmias of thyroid origin cause death?

With regard to the first question, it has been stated that cardiac pain occurs rarely in thyrotoxicosis (4, 5) In a study of 1 000 patients with thyrotoxicosis Ernstene (8) found only 5 with angina pectoris, and Summers and Surtees (21) found an incidence of 4.5% in their survey of 200 patients with hyperthyroidism. However others, reviewed by Burstein et al. (4) have found incidences up to 20% More recently Rohrbach (18) reported 11 patients with masked hyperthyroidism, 4 of whom complained of cardiac pain, and Sandler and Wilson (20), in their comprehensive review of 462 patients with thyrotoxicosis, had 21 patients with angina pectoris and 22 others with gross ECG evidence of ischemic heart disease or previous infarction. These differences in the incidence of cardiac pain probably reflect differences in the age and sex distribution of the reported series, as was clearly the case in the survey of Sandler and Wilson. It is to be expected that cardiac pain, due to concomitant ischemic heart disease will occur more frequently in older than in younger patients with thyrotoxicosis, although the course of coronary bouts may be distinctly mild in patients in the thyrotoxic state (4) That severe pain, mimicking coronary occlusion, may however occur in patients with thyrotoxicosis in whom subsequent autopsy studies show no evidence of coronary disease is attested to by several reports (7 17). With regard to the present case it would appear justified to conclude that angina pectoris and more severe cardiac pain, mimicking coronary occlusion, may occur in patients with thyrotoxicosis, even though no acute infarction is subsequently found.

With regard to the second question, it is well known that by far the most common type of cardiac arrhythmia in thyrotoxicosis is atrial fibrillation. Ernstene (8) found this type of arrhythmia in 207 of 1 000 patients with thyrotoxicosis, Bourel et al. (7) in 24 of 83 such patients, Rohrbach (18) in 4 out of 11 cases of masked thyrotoxicosis, while Sandler and Wilson (20) found an overall number of 84 in 462 patients with thyrotoxicosis. The incidence of this type of arrhythmia therefore appears to be about 20% Other arrhythmias occur much more rarely Thus Ernstene among 1 000 patients with thyrotoxicosis, found 2 with atrial flutter 5 with auricular fibrillation and 2 with ventricular tachycardia, while Rohrbach noted premature ventricular beats in 1 of 11 patients. Prolonged A–V conduction time as a cardiac complication of thyrotoxicosis is mentioned by Cookson (5) and Blizzard and Rupp (1) have reported an increased P–R interval in 5 of 76 thyrotoxic patients. At one time or another the patient under discussion presented all of these types of arrhythmia, although treatment with digoxin and a β-blocking agent may hav contributed to the transient episode of A–V dissociation, which precipitated his transfer to this hospital It can be concluded that ventricular tachycardia, to an episode of which the patient succumbed, may occur in rare instances in thyrotoxicosis.

The final question, whether thyroid-induced cardiac arrhythmias may be the cause of death, would therefore appear to be answered in the affirmative by the present case history To our knowledge there are no previous reports on such an event, which must be extremely rare. Wolfson and Smith (25) have, however reported cardiac arrest following minor surgery in a 12 year-old girl with unrecognized thyrotoxicosis. The ECG showed ventricular fibrillation which was converted after several attempts with DC counter shock. In their case the authors suggest a combined effect of halothane anesthesia and thyroid storm in precipitating the episode of entricular fibrillation.

The mechanisms whereby thyroid hormones exert their effects on the heart appear to be complex. For number of years attention was focused on the interrelations between thyroid hormones and catecholamines (11 23) The similarities between the hemodynamic effects of excess thyroid hormone and of epinephrine administration have in recent years prompted a number of studies on the effect of sympathetic blockade on the symptoms of hyperthyroidism.

These studies, e.g. with guanethidine (9) and propranolol (12), have demonstrated that, although sympathetic blocking agents do indeed diminish many of the hemodynamic abnormalities characteristic of the thyrotoxic state a complete return to normal conditions is not achieved (13). Thyroxin also therefore appears to have a direct effect on both cardiac rate and contractility a conclusion already reached from experiments 40 years ago (15 26) and confirmed more recently by Thier et al. (22) and Buccino et al. (3). Direct biochemical evidence in support of an action of thyroxin on the heart, independent of catecholamines, has recently been presented by Levey and Epstein (14) who were able to demonstrate the presence in cat heart homogenate of two adenyl cyclase systems, one activated by catecholamines, the other by thyroid hormones. Only the adenyl cyclase system, activated by catecholamines, could be inhibited by the β-blocking agent propranolol.

Finally thyroxin excess has a more indirect effect on the heart by increasing the oxygen requirements of the tissues and thereby the circulatory load. As shown by Graettinger et al. (10), the increase in circulatory load may be of such a magnitude as to overcome the reserve of he otherwise normal heart, leading to high out t cardiac failure

From what has been discussed above, it is apparent that adequate treatment of thyrotoxicosis, accompanied by one or more of the cardiac complications mentioned, may present a therapeutical challenge This applies in particular to the treatment of thyroid storm (6 16) Dillon et al. (6) have reported the successful treatment of this condition with reserpine, while Mazzaferri and Skillman (16) demonstrated an improvement in survival to 93 % in 20 patients treated with guanethidine over the past 10 years. As pointed out in a recent review (19) it is important not only to counteract the acute effects of catecholamines on the circulatory system, but also to reduce the excess production of thyroid hormones as quickly as possible by treatment with large doses of antithyroid drugs and in particular to inhibit the release of thyroxin from the over active gland with iodine (24).

In conclusion it seems warranted to emphasize that the diagnosis of thyrotoxicosis should be considered in all less characteristic cases of cardiac arrhythmia, despite the occurrence of precordial pain suggesting AMI

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INTRINSIC FACTOR SECRETION FOLLOWING PENTAGASTRIN AND INSULIN STIMULATION IN DIABETES MELLITUS

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Abstract. In 10 patients with diabetes of long duration, most of them exhibiting late manifestations of the disease, and in a control group the intrinsic factor secretion has been studied after stimulation with pentagastrin and insulin. The responses to pentagastrin were similar in the two groups, indicating that there was no serious destruction of the gastric mucosa. On the other hand the secretory response differed after insulin stimulation. In the diabetic group the response was about 4/10 of the pentagastrin response, the corresponding proportion in the control group being about 9/10. This discrepancy is interpreted as a manifestation of diabetic neuropathy

It is possible that an autonomic neuropathy in diabetes mellitus affects every function of the organism that is under autonomic control. Perspiration abnormalities, orthostatic hypotension and probably gastrointestinal disturbances and bladder dysfunction may all have this common cause. The vasomotor abnormalities have been well documented by clinical physiological studies (12). The visceral signs and symptoms are motor weakness and hypotension which are attributed to diabetic neuropathy. Pathoanatomical changes have, however not been convincingly demonstrated in the autonomic nervous system at either biopsy or postmortem examination. Kassander (8) described the gastric atony and ascribed it to autonomic neuropathy in the same way as hypomotility following vagotomy (6).

We have earlier discussed the vagally induced hydrochloric acid production of the stomach (5). With the advent of immunological methods, several methods for determining intrinsic factor have become available. The present investigation was designed to study the intrinsic factor responses to humoral and vagal stimulation in patients with diabetes mellitus

MATERIAL

The investigation was performed on 10 [illegible] th diabetes of varying duration (15.0 ± [illegible] S.D.) and 10 control subjects. The patients [illegible] trols were of similar age 29.3 ± 7.0 and [illegible] respectively. The patients were all on [illegible] diet consisting of 40% carbohydrates, [illegible] 30 fats. At the time of observation [illegible] clinically satisfactory metabolic state [illegible] patients exhibited some form of late diabetic [illegible] such as nephropathy and/or varying [illegible] pathy and or neuropathy. Seven [illegible] neuropathy. None manifested signs [illegible] the gastrointestinal tract. The controls [illegible] good health and had no diet [illegible]

PROCEDURE AND [illegible] METHODS

Gastric secretory studies were perfor[illegible] after 12 hours' fasting and [illegible] Before the study all patient [illegible] of insulin. A nasogastric tube [illegible] introduced.

The patient was in semi[illegible] structed not to swallow saliva [illegible] stomach was carried out by [illegible] subatmospheric pressure [illegible] Injection of air and intermittent [illegible] syringe was used to prevent [illegible] juice was pooled for individual [illegible] lowing tests were performed

Pentagastrin test. Basal [illegible] 1 hour. Thereafter pentag[illegible] subcutaneously in [illegible] collection of aspirates was [illegible]

Insulin test. When the [illegible] samples of capillary blood [illegible] determination by the [illegible] order to get immediate [illegible] was approximated [illegible]

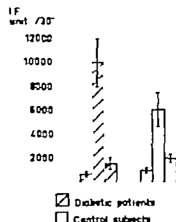


Fig. 1

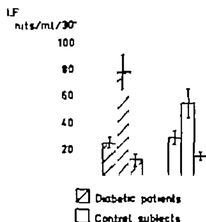


Fig. 2

Figs. 1 and 2. Intrinsic factor output and concentration (mean ±S.E.), basal and after pentagastrin. Each column represents half an hour

Ames, England) which were used simultaneously with the glucose-oxidase method. After 60 min collection of basal secretion and determination of blood sugar value 20 I.U of insulin (Insulin, Vitrum, Stockholm, S eden) was given I. to the controls. Between 16–32 I.U as given to the patients, depending on the initial blood sugar value. The secretion was followed for 2 hours and the blood sugar value was determined every 30 min. Clinical signs of hypoglycaemia were seen in all patients and controls.

Intrinsic factor activity as determined by the method by Ardeman and Chanarin (2) using ^{57}Co-labelled B_{12} as the radioactive isotope. Intrinsic factor assayed as the difference between total vitamin B_{12} nding capacity and vitamin B_{12} binding capacity after complete blockage of intrinsic factor by an excess of antibodies to intrinsic factor. An intrinsic factor unit is defined as the specific intrinsic factor binding 1 ng vitamin B_{12}.

Conventional statistical methods have been used, including Student's *t*-test for testing the significance of differences between the groups. A *p* value of <0.05 is considered significant.

RESULTS

As appears from Figs. 1 and 2, the patients tended to attain both a higher mean output and a higher concentration of intrinsic factor in response to pentagastrin stimulation than the controls, although no statistically significant differences were noted ($p > 0.05$).

The mean intrinsic factor output in response to insulin-induced hypoglycaemia in the diabetic group was lower during the first hour ($p > 0.05$).

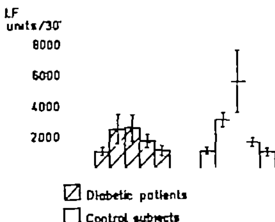


Fig. 3

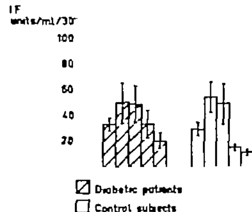


Fig. 4

Figs. 3 and 4. Intrinsic factor output and concentration (mean ±S.E.), basal and after insulin. Each column represents half an hour.

but the difference cancelled out during the second. Figs. 3 and 4 demonstrate that the intrinsic factor concentration was practically identical in the two groups after stimulation by insulin-induced hypoglycaemia.

When half the 2-hour response to hypoglycaemia is divided by the 1-hour pentagastrin response for each person separately it appears from Table I that the intrinsic factor production in the two groups differed significantly. While the intrinsic factor response to insulin hypoglycaemia in the control group attained nearly 9/10 of the pentagastrin response, the corresponding value for the diabetic group was merely 4/10 ($p < 0.05$).

The blood sugar levels initially and after insulin in the two groups are shown in Fig. 5.

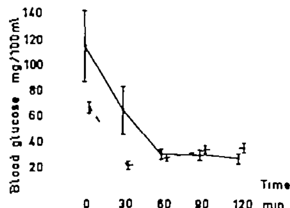


Fig. 5. Blood sugar levels (mean $\pm$ S.E.) for the diabetic patients (——) and for the controls ( ) before and during insulin hypoglycaemia.

DISCUSSION

It is suggested that intrinsic factor is stored in the gastric mucosa. Intrinsic factor estimations have been used mainly in the diagnosis of pernicious anaemia and for evaluation of atrophic gastric lesions. When the stomach is stimulated the intrinsic factor concentration rises in the gastric juice. After subcutaneous histamine or pentagastrin doses an initial output of intrinsic factor is observed before either acidity or volume of gastric juice have peaked (14). Jeffries and Sleisinger (7) and Weir et al. (14) proposed that the first phase represents a wash-out phenomenon of preformed intrinsic factor stored in the mucosal cells. This phase is followed by less rapid secretion which represents the rate of intrinsic factor production in the gastric mucosa. Bitsch et al. (4) reported that during the first hour after insulin stimulation the intrinsic factor output in patients with peptic ulcer was about the same as after augmented histamine stimulation, although there was some suppression during the first quarter hour and a peak during the third quarter.

The aim of the present investigation was to study the secretory pattern of intrinsic factor after humoral and vagal stimulation. Only few papers have been published on the secretion of intrinsic factor after histamine stimulation, still fewer deal with intrinsic factor studies after insulin and pentagastrin stimulation in young, healthy subjects. Shearman et al. (13) tested a series of 15 patients with gastric distress and found that pentagastrin elicits a gastric intrinsic factor response resembling that following augmented histamine stimulation. On the other hand Rødbro and Christiansen (11) using a modified charcoal-serum method for determination of intrinsic factor obtained higher basal values in healthy young men and higher output after histamine stimulation during the first hour than in our pentagastrin-stimulated control subjects.

The accepted method of checking continuity of the vagal innervation of the stomach is to measure the gastric acid response to insulin hypoglycaemia. Cerebral hypoglycaemia stimulates the vagal nuclei and this, by way of the vagal nerve,

Table I. *Insulin response/pentagastrin response of intrinsic factor*

Diabetic patients Case no.	%	Controls Case no.	%
1	24	11	72
2	11	12	201
3	42	13	180
4	33	14	72
5	73	15	39
6	53	16	107
7	66	17	40
8	29	18	51
9	10	19	27
10*		20	66
Mean	38		86
S.D.	23		60

* Excluded.

stimulates the stomach to produce more acid. The insulin test is used before and after surgical vagotomy to study how complete the vagotomy is. So far however no laboratory criteria seem to have been defined whereby complete surgical vagotomy can be distinguished from incomplete. Numerous variations of Hollander's original insulin test have been proposed by different authors, most of them demanding blood sugar levels below 50 mg/100 ml in order to ensure a maximal stimulus. This corresponds to 40 mg/100 ml when our method for blood sugar determination is used.

In the present investigation we compared the insulin- and pentagastrin-induced gastric intrinsic factor responses. The laboratory criteria for blood sugar levels were the same as used in the insulin test for gastric acid production. (One patient with diabetes mellitus was eliminated from the series on account of absence of a secretory response to pentagastrin stimulation.) A sudden drop in a high blood sugar value is not a stimulus to gastric secretion but may be an additional stimulus only when the blood sugar level is about or below 50 mg/100 ml (3). The patients received their ordinary dose of insulin in the morning; and when the examination began a few hours later all the patients had a blood sugar level above 50 mg/ ml, which excludes a wash-out of intrinsic before the beginning of the examination. On the whole the patients with diabetes mellitus started with a higher basal blood sugar level. In the first 30 min it had not been reduced sufficiently to ensure maximal vagal stimulation in about half the group, blood sugar levels below 40 mg/100 ml being recorded only after 1 hour. Accordingly we preferred to compare half the intrinsic factor output during 2 hours insulin stimulation with the pentagastrin-stimulated intrinsic factor output during 1 hour in order not to lose any late wash-out of intrinsic factor in the patients with diabetes mellitus.

The secretory intrinsic factor response to pentagastrin was of the same order in the two groups, implying that the gastric mucosa cannot have been seriously damaged in the diabetic group. Conversely the secretory responses to insulin were dissimilar in the two groups. During the first hour the output was lower in each sample from the diabetic group, but this difference on the whole cancelled out after 2 hours.

In the diabetic group the intrinsic factor response to insulin stimulation was about 4/10 of the response to pentagastrin. The corresponding proportion in the control group was approximately 9/10. This difference in intrinsic factor activity is significant. It is mainly due to reduced volume in the diabetic patients and not to reduced concentration.

In the control group the gastric acid response (9) to insulin-induced hypoglycaemia was about $87 \pm 23\%$ of that to pentagastrin-induced, and the corresponding figure for intrinsic factor was $86 \pm 60\%$ suggesting that the vagal contribution to the regulation of intrinsic factor secretion is comparable with the vagal contribution to the control of acid output. In the diabetic group the corresponding proportions for acid and intrinsic factor were $49 \pm 30\%$ and $38 \pm 23\%$ respectively. Consequently this study gives support to the theory that acid and intrinsic factor are reduced in the same magnitude by disturbance of the vagal function. Adams et al. (1) expressed the view that medical vagotomy does not affect the intrinsic factor reduction in the same way as surgical vagotomy. They proposed that the vagal component in the control of intrinsic factor secretion might be less dominant than the vagal component in the regulation of acid output.

The present investigation is interpreted as showing that, in a small group of patients with diabetes of long duration but without gastrointestinal disturbances, the gastric intrinsic factor response to insulin-induced hypoglycaemia is distinctly reduced. This is thought to be a manifestation of diabetic neuropathy involving the vagal nerve.

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EFFECTS OF PHYSICAL TRAINING ON GLUCOSE TOLERANCE, PLASMA INSULIN AND LIPIDS AND ON BODY COMPOSITION IN MEN AFTER MYOCARDIAL INFARCTION

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Abstract Every second of non-selected men below the age of 55 years who had survived myocardial infarction was placed in group for physical training, while each other constituted a control. The physical training program was selected according to each patient' individual maximal orking capacity and lasted for 9 months, beginning 3 months after the myocardial infarction. At the end of the training period the experimental group was subdivided into one group of adequately trained patients and one consisting of drop-outs and patients with poor adherence. The patients who followed the program increased their physical work performance. In this group a decrease was found in plasma insulin values after glucose tolerance test, slight increase in glucose tolerance and decrease in body fat and plasma triglycerides. Body cell mass and plasma cholesterol did not change. The men selected for the training program, but who were not able to follow it sufficiently as well as the controls, showed less marked decrease in plasma insulin values at reexamination.

In relation to the large number of studies concerned with effects on plasma lipids of changes in the quality and quantity of caloric intake the interest in the effects of energy output changes has been much less. In healthy individuals physical training apparently produces minor or no changes in plasma cholesterol (6, 11 18 19 20 25 29) while plasma triglycerides are lowered to 22–40% (14 18 29). This decrease is of the same order as that observed after acute prolonged exercise (8). In patients with cardiovascular disease plasma cholesterol has been found to decrease moderately after physical training (27) The present paper describes the effect of physical training on body composition, plasma insulin and lipids, and glucose tolerance, in men who have survived a myocardial infarction.

MATERIAL AND METHODS

The material of myocardial infarction patients has been described in detail previously (2). Briefly it consists of all men below the age of 55 years ho have suffered myocardial infarction and survived in the city of Gothenburg, Sweden, from 1 January 1968 through 31 May 1969 E ery second of these 104 men was selected for physical training program. The remaining 52 patients constituted the non-training group.

When discharged from the hospital after the myocardial infarction the patients were given similar instructions. They were recommended to avoid bed rest. Obvious dietary abnormalities were corrected by dietician towards a diet similar to S edish normal diet (5).

At the beginning of the training period repeated exercise tests ere performed on an electrically braked bicycle ergometer with stepwise increasing ork loads, 4 min at each step, up to maximal" xercise. The exercise was interrupted due to general fatigue, angina pectoris, ECG changes, poor BP regulation or locomotor symptoms. Exercise tests were also performed during the training period in order to direct the training intensity One year after the myocardial infarction all patients were reexamined. The training consisted of an interval program of cycling, running and calisthenics for / hour three times week during 9 months under the supervision of physiotherapist and physician. The heart rate during the heavy intervals was on an average 146 ± 16 beats/min (range 109–164). On the day determined for examination of the effects of the procedures (31 May 1969) 32 of the patients originally selected had been reexamined one year after the infarction. Only 15 of them had adhered adequately to the physical training program and had reached the prescribed pulse rate during the different exercises. The remaining 17 could not follow the program in sufficient way The physical ork capacity—in terms of reduced heart rate at submaximal work load or the maximal achieved ork load (Table I)—increased significantly in the first mentioned group, while this as not the case for the group hich did not follow the training program sufficiently At the same date 26 of the

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ADDISON'S DISEASE IN A UNIVERSITY MEDICAL DEPARTMENT DURING 20 YEARS

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Abstract. In the Medical Department of the University Hospital in Uppsala 26 instances of Addison disease have been observed during the years 1949–68. The yearly intake of patients was some 3 000 with an upward trend. This material is briefly reviewed with regard to etiology and symptomatology

Suprarenal disorders are, on the whole rare in a university medical department which, like ours in Uppsala, is not differentiated in various subspecialities. Personally we remain convinced of the advantages, not only for science but for medical education as well, of keeping the internal medicine integrated into one unit.

We have during the years 1946–68 seen about one dozen cases of pheochromocytoma, some few examples of Conn's tumor and also a certain number of Cushing's syndrome. As for the minus-conditions we have during the last two decades registered 26 instances of Addison's syndrome.

ETIOLOGY

Out of 26 cases 8 were males, 18 females. Etiologically at least 11 (4 males, 7 females) were caused by tuberculosis. Four instances were considered iatrogenic (in 2 females removal of the suprarenals in order to inhibit the malignant metastases, in 2 other females prolonged treat ment with cortisone preparations because of rheumatoid arthritis) In one male case there was a thrombosis of the inferior vena cava and it is possible that this involved the suprarenal veins as well. In the rest of the material (3 men, 7 women) the etiology was unknown and presumed to be a primary atrophy" or autoimmune disease or in one case possibly tuberculosis as well This was a man who had had tuberculous spondylitis and had to work in a foundry it is difficult to imagine an occupation less well suited for a patient with Addison's syndrome.

The duration of the disease was in the male cases less than 1 year in 3 instances, 3– 8 years in 5 Of the females 4 had had symptoms for less than 1 year the rest for 1–17 years We have intentionally excluded our first 3 years in Uppsala when we had no cortisone. Also 2 instances of apoplexy in the suprarenals, both fatal, have intentionally been excluded.

DIAGNOSIS

The diagnosis was established beyond reasonable doubt by means of 1) the characteristic clinical syndrome,) the laboratory tests, 3) the results of the treatment, 4) calcification of the suprarenals (2 males, 2 females) 5) the necropsy (2 males, 6 females) 6) the surgical removal of the suprarenals (2 females)

SYMPTOMATOLOGY

The clinical syndrome was characterized by symptoms of fatigue dyspepsia and abdominal pain and by signs of weak oice increased pigmentation and low blood pressure. A marked craving for sodium was noticed in at least 2 cases and might have been present in more.

It should be emphasized that a clinical syndrome masquerading as Addison s disease may be represented by conditions impairing the intestinal absorption, e.g. a severe steatorrhea or a fistula between the duodenum and colon However In these cases there is a feature alien to Addison's disease: a decreased protein level of the plasma (1).

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It is well known that the EEG may be abnormal in Addison's disease. It has even been maintained that choked discs might possibly be present. We have failed to convince ourselves of this, and yet there is no case in our department during the years reported here in which ophthalmoscopy had been omitted. On the other hand, mental irritability as well as confusion may be observed mostly during a crisis. Muscular fatigue frequently involving all four extremities, is frequently found, particularly in females during the days preceding their periods or at the end of a day. It should be observed, however that weakness of the legs may be observed in other endocrine disorders. Such is the case e.g. in Conn's syndrome: it is frequently impossible to climb up on a chair at least during the days preceding the periods. In thyreotoxicosis it is well known that the ensuing myopathy may result in a similar inability to walk upstairs without the support of the hand on the rail.

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HEMODYNAMIC EFFECTS OF NOREPINEPHRINE IN SEVERE HYPNOTIC DRUG POISONING WITH ARTERIAL HYPOTENSION

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Abstract. The hemodynamic response to infusion of norepinephrine (NE) has been measured in 12 patients with severe hypnotic drug poisoning. All patients had mean arterial blood pressure (B.P.) of 70 mmHg or less initially. As evaluated from central venous pressure no marked hypovolemia was present. Cardiac output (C.O.) was determined by the dye dilution method and right heart catheterization by "floating" catheter technique. Following infusion of NE the arterial B.P. and C.O. increased. The increase in C.O. resulted from increases in stroke volume and to a less extent also in heart rate (HR). There was no significant change of peripheral vascular resistance index (PVRI), in contrast to the NE effect in cardiogenic, hemorrhagic and septic shock states. The increase in arterial B.P. was accompanied by an increase in HR, which implies a disturbed function of the high pressure baroreceptor system. The rise in B.P. is primarily due to the increase in C.O. No adverse effects were noted during the NE infusion. It is concluded that NE is beneficial in the treatment of severe hypnotic drug poisoning complicated by hypotension.

Severe poisoning by hypnotic drugs results in coma and hypothermia. This condition is often complicated by arterial hypotension and hypoventilation. Depression of the central nervous system with faulty regulation of the peripheral vascular system has been considered one of the dominating mechanisms. It is also known that barbiturates and other sedative drugs have a depressive effect on myocardial contractility (6, 9, 13). When barbiturates are given in moderate doses this effect can be counteracted by sympathomimetic amines (4, 9). Shubin and Weil (32) reported that patients with severe hypnotic drug poisoning may have a reduced plasma volume. However, the degree of hypovolemia may be slight and is not likely to be the only explanation of the hypotension.

In hemorrhagic shock norepinephrine (NE) further reduces peripheral blood flow and is therefore usually contraindicated (26). In cardiogenic shock it may have beneficial effects in some cases (18, 22). In severe hypnotic drug poisoning the depression of the central nervous function probably also implies a reduced sympathetic outflow, resulting in disturbed regulation of circulatory functions. Thus it seems logical to use the transmitter substance of the sympathetic nervous system, NE, in the treatment of hypotension in these patients. On empirical grounds NE and related drugs have also been used successfully (8, 10, 12, 30). However, only few studies have been reported on the circulatory effects of NE in such patients. Shubin and Weil (32, 33), who stress the importance of hypovolemia, report beneficial effects of NE and metaraminol given in small doses to some patients, but in general NE is not recommended because a high mortality rate was observed in a group of patients treated with it. This is contradictory to our own clinical experience.

In a recent study circulatory dynamics at various stages during the course of poisoning by hypnotic drugs have been reported (3). The present study was undertaken with the aim to evaluate the hemodynamic effects of treatment with NE in patients with hypotension because of severe hypnotic drug poisoning.

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Table I. *Some anthropometric and other data for 12 patients with drug poisoning*

Case no.	Sex	Age (y)	Height (cm)	Weight (kg)	BSA (m^2)	Type of intoxication	Duration of coma (h)	Min. body temp. (°C)
1	♂	71	169	75	1.85	Butenemal-allypropymal (Dimiual duplex)+alcohol	70	31 1
2	♀	71	169	65	1 71	Mebumal (Nembutal)	60	30.4
3	♀	56	165	57	1.63	Meprobamate	35	36.8
4	♀	30	162	45	1 45	Amitryptiline (Tryptizol®)	60	25.8
5	♀	68	166	46	1 48	Glutethimide (Doriden®)	95	32.0
6	♀	40	152	48	1 42	Diazepam (Valium®)+ meprobamate	70	32 0
7	♀	72	164	46	1 48	Butenemal-allypropymal (Diminal duplex®)	90	31.9
8	♀	72	162	70	1 75	Carbamazepine (Tegretol®)	205	29 1
9	♂	54	180	80	2.10	Butenemal-allypropymal (Dimiual duplex®)	85	32.5
10	♀	30	164	64	1 69	Fenemal	75	33.8
11	♂	50	170	69	1.81	Amitriptylin (Tryptizol®)+ glutethimide? (Doriden®)	70	31.3
12	♀	71	165	69	1 76	Butenemal-allypropymal (Diminal duplex®)	145	32.6

MATERIAL

Twelve patients, three men and nine women, have been investigated. Their mean age was 53 years (range 24–72). Anthropometric data, drugs used and severity of the poisoning are given in Table I. All patients were deeply comatous following the ingestion of high doses of hypnotic drugs. In the sequel sedative and antidepressive drugs will be included in the term hypnotic drug. Patients admitted because of drug poisoning have often taken a combination of drugs and it may be difficult to identify exactly the various drugs used. In spite of this the clinical picture as well as the hemodynamic disturbance is remarkably similar as recently reported (2). It is therefore justified to regard the material as relatively homogenous from a hemodynamic viewpoint, although this is not true on a strictly pharmacological basis. Body temperature was on the average 33.8°C during the hemodynamic measurements (range 28.8–37 7°C). Only patients with a mean arterial B P of 70 mmHg or less without infusion of NE were included.

Supporting treatment included assisted ventilation with oxygen added if there were signs of hypoxia (Engström Respirator). All patients received NE before and after the study Parenteral dextrose-electrolyte solution was administered simultaneously Urinary output was adequate in all patients. Digitalis (case 3) and diuretics were given to some patients. One patient (no. 8) had intracardiac pacing by means of a transvenous catheter electrode because of atrial fibrillation with pronounced ventricular bradycardia, but fulfilled the other criteria and was therefore included. One patient (no. 12) underwent cardiac resuscitation because of heart standstill on arrival at the ward. This patient died from pulmonary edema and renal insufficiency two days after the investigation Another patient (no. 11) died after 12 hours. The probable cause of death was hypoxia as a result of pulmonary edema and atelectasis.

METHODS

On admission serum was analyzed for barbiturates, urine for phenothiazine derivatives and meprobamate. Analyses for other drugs were also made in some patients (Table I)

All patients were studied at the bedside in supine position. Central venous pressure was measured through an indwelling teflon catheter inserted into the right or left subclavian vein. Through this tube a fine polyethylene catheter (PE 60) connected to a pressure transducer was allowed to float with the blood stream into the pulmonary artery (7) The position of the catheter tip was determined by observation of the pressure curve. A teflon catheter was inserted into the femoral artery Pressures were recorded on a direct writing ultraviolet 10 channel recorder (ABEM Ultralette). The reference point for zero pressure was taken at the mid-thoracic level. Mean pressures were obtained electronically Cardiac output (C.O) was determined by dye dilution technique using indocyanine green. Dye was injected as a bolus into the pulmonary artery and blood was drawn from the femoral artery through a Beckman Cardio-Densitometer at a constant flow of 20 ml min using a 50 ml glass syringe adapted to a withdrawal pump. The blood was reinfused. The areas under the dye dilution curves were calculated by means of a built-in disk integrator and logarithmic extrapolation.

Systemic vascular resistance index was calculated as the quotient between mean femoral artery pressure minus central venous pressure and cardiac index. Left ventricular stroke work was calculated from the following formula: mean arterial pressure pulmonary artery diastolic pressure (mmHg) 13.6 stroke volume (ml) and expressed in GmM by dividing by 1 000.

Arterial and mixed venous blood were sampled simultaneously and the O_2 content was determined spectrophotometrically (Beckman B spectrophotometer). Arterial PO_2, pH and PCO_2 were measured by electrode technique (Radiometer BMS 3). The ECG was monitored throughout the investigation. NE was diluted in 0.9% NaCl solution and administered by use of Harvard automatic infusion pump. Doses ranged from zero to 0.79 $\mu g\ kg^{-1}\ min^{-1}$. Administration of NE was discontinued for at least 15 min before basal hemodynamic measurements were carried out, except in four patients (nos. 1, 5, 8 and 12), who required small dose of NE to keep mean B.P. above 50 mmHg. In order to obtain steady state, each dose level was maintained during at least 15 min before measurements were carried out.

RESULTS

Cardiac output and arterio-venous oxygen difference. Following infusion of NE, C.O. increased from an average of 3.6 l/min initially (range 1.8–7.8) to 5.1 l/min (range 2.4–8.6) with the highest dose rate (Fig. 1, Table II). This increase was significant ($p < 0.001$) and amounted on the average to 1.5 l/min. In nine patients the arterio-venous oxygen difference was determined. It decreased significantly ($p < 0.01$) following administration of NE, but since the relative increase in C.O. was more pronounced the calculated oxygen consumption increased slightly.

Heart rate (HR). Initial values varied considerably (40–122). After NE there was an increase in eight patients and a decrease in three (Fig. 1). Patient 8 was treated with pacemaker. With this patient excluded the increase, however, was not significant.

Stroke volume. The initial value for the stroke volume averaged 47 ml (range 22–74). With infusion of the highest dose of NE it increased in all patients but one, in whom the stroke volume was unchanged (Fig. 1). The change was significant ($p < 0.001$) and averaged 16 ml (range 0–44).

Intravascular pressures. With increasing dose rate of NE femoral artery pressure increased in all patients (Fig. 2). The average initial mean arterial pressure was 60 mmHg (range 50–70). Following NE at the highest dose rate it increased to 89 mmHg (range 69–115). The mean pulmonary arterial B.P. was normal or slightly elevated initially. Following infusion of NE there was a small but significant increase ($p < 0.001$). The diastolic pulmonary artery pressure was slightly elevated in two cases (nos. 2 and 3) during the initial determination. There was a probably significant increase ($p < 0.05$) of diastolic pulmonary artery pressure following infusion of NE.

Peripheral vascular resistance index (PVRI). The mean PVRI was the same in this material (31 U) as in a control group (30 U) of about the same mean age (23). There was no significant change of PVRI following infusion of NE (Fig. 3).

Stroke work. Following infusion of NE there was a pronounced increase of left ventricular stroke work which was accompanied by a slight increase in pulmonary artery diastolic pressure (Fig. 4).

Arterial blood gases and acid-base balance. Arterial PO_2 was low in several cases despite addition of oxygen to the inspiratory gas. In cases 4, 6 and 12 arterial PO_2 was very low. There was generally a rise of PaO_2 and a decrease of $PaCO_2$ following NE administration, but the changes were not significant. Base excess, however, decreased significantly ($p < 0.01$). In several cases there were signs of hyperventilation which was generally induced by the respirator treatment. This compensated for a tendency to metabolic acidosis in a few cases. One patient had a marked metabolic alkalosis.

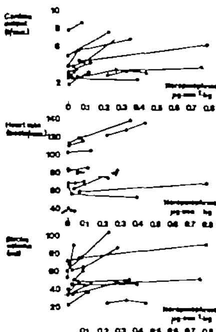


Fig. 1. Cardiac output, heart rate and stroke volume in relation to NE dose rate in 1... patients with hypotension from poisoning with hypnotic drugs. ... without, ● = with NE.

Table II Hemodynamic data following administration of NE to 12 patients with hypnotic drug poisoning and hypotension

CV = central venous, BE = base excess, FA = femoral artery PA = pulmonary artery S = systolic, D = diastolic, M = mean

Case no.	Body temp. (°C)	Dose rate (NE µg min⁻¹ × kg⁻¹)	HR (beats/min)	C.O (l/min)	AV-O_2 diff (ml/l)	Stroke volume (ml)	Pressures (mmHg) CV	FA S	FA D	FA M	PA S	PA D	PA M	PVRI (U)	PaO_2	PaCO	BE (mEq/l)	pH
1	32.8	0.06	59	4.4	—	74	2	90	39	56	19	7	13	23	59	46	+3	7.41
	33.0	0.79	69	6.3	—	91	4	146	50	82	23	11	18	22	—	—	—	—
2	36.3	—	110	7.8	—	71	5	100	56	70	26	17	20	14	59	29	−2	7.46
	37.0	0.08	115	8.6	—	73		126	76	86	30	18	25	17	49	26	−1	7.45
3	36.0	—	112	3.8	—	34	9	70	50	59	27	15	20	21	67	36	−6	7.35
		0.06	118	5.6	—	47	6	92	64	80	25	14	19	22	71	34	−9	7.31
	37.2	0.35	139	6.9	—	50	9	105	75	92	36	23	29	20	77	36	−7	7.33
4	28.8	—	82	1.8	64	22	5	91	55	69	17	10	12	52	40	19	0	7.42
	30.2	0.11	86	3.2	40	37	3	145	93	115	17	6	12	51	84	22	−7	7.40
5	37.6	0.03	62	3.8	47	61	4	67	36	50	19	7	13	18	59	37	−3	7.39
		0.09	67	4.3	38	64	6	93	52	70	1	11	17	22	51	34	−4	7.39
	37.7	0.27	83	7.1	—	86	6	148	74	106	35	15	24	1	51	34	−5	7.38
6	33.6	—	93	3.2	44	34	4	82	43	55	21	13	15	23	31	22	+4	7.43
	34.1	0.13	95	4.4	38	46	5	98	64	69	[illegible]	14	19	21	29	21	+3	7.44
7	32.6	—	64	2.5	34	39	5	95	46	65	4	11	16	36	78	33	−2	7.44
	32.0	0.02	56	2.5	39	45	5	118	51	79	24	11	16	44	98	26	−5	7.45
	33.2	0.39	53	2.4	—	45	5	141	61	91	31	13	19	53	114	32	−6	7.49
8	29.3	0.05	79	[illegible]	40	34	9	79	44	54	26	14	17	30	43	33	−3	7.41
	30.0	0.27	79	3.5	34	46	7	120	57	78	30	11	17	36	53	25	−2	7.52
	29.8	0.76	78	3.8	36	51	6	128	59	80	30	12	16	34	65	24	−6	7.47
9	32.5	—	40	[illegible]	57	73	3	104	58	70	18	7	11	49	49	23	−1	7.55
	[illegible]	0.01	37	[illegible]	39	78	3	125	65	83	21	8	13	55	83	22	−2	7.53
	32.8	0.03	36	3.2	53	89	7	158	74	99	26	12	17	60	94	22	−4	7.51
10	35.3	—	84	5.0	39	60	5	107	53	67	18	7	12	21	52	44	+15	7.57
	36.0	0.23	74	7.7	31	104	6	118	59	76	23	10	16	15	70	46	+10	7.49
11	31.5	—	68	2.7	34	40	6	72	49	55	18	9	12	33	136	33	+7	7.55
	31.7	0.04	68	3.4	31	50	6	108	64	75	24	11	16	37	110	21	−4	7.52
	31.8	0.09	70	4.2	22	60	4	126	72	84	28	11	18	34	125	26	0	7.52
12	36.1	0.22	122	2.9	59	24	4	77	47	57	22	11	15	32	39	34	+2	7.48
	36.3	0.33	128	3.4	51	27	5	117	64	82	26	11	16	40	45	31	−3	7.43
	36.5	0.44	134	3.2	50	24	4	135	75	95	26	12	17	50	44	30	−4	7.42

DISCUSSION

Patients who were unable to maintain a mean intraarterial B.P. above 70 mmHg were selected for the present study. The mean age of the present material is higher than that of all patients admitted because of hypnotic drug poisoning. This indicates that hypnotic drug poisoning is more often complicated by severe arterial hypotension in old patients than in young. Disregarding one patient (no. 12) who had systemic hypertension, none of the patients had a history of previous heart disease. Two patients (nos. 7 and 8) showed reversible ECG changes with depression of ST and T segments corresponding to the left ventricle. Cases 4 and 11 showed widening of the QRS complex, indicating poisoning with a tricyclic antidepressant drug. No further changes were observed during the infusion of NE. Despite high doses of NE no ectopic ventricular beats were observed in any of the patients.

The low arterial oxygen tension values could be caused by pulmonary vascular congestion or shunting. Cases 2, 11 and 12 had signs of pulmonary congestion on bedside X-ray films. In case the diastolic pressure in the pulmonary artery was slightly elevated. In the present material no correlation between lowered arterial oxygen tension and pulmonary artery pressure could be observed. The hypocapnia due to hyperventilation may have decreased C.O. in three patients (7) and probably increased the sensitivity to NE following changes in pH (34). It is unlikely that the slight metabolic acidosis and lowered pH in patient 3 could have added to the hypotension in this patient.

The hemodynamic effects of hypnotic drug

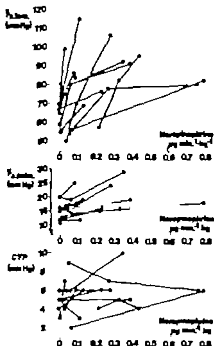


Fig. 2. Mean femoral artery, pulmonary artery and central venous pressures in relation to NE dose rate. Symbols as in Fig. 1.

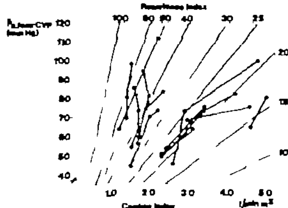


Fig. 3. Mean femoral artery minus central venous pressure in relation to cardiac index. Isoresistance lines are indicated. Symbols as in Fig. 1.

poisoning are characterized by a low C.O., usually a moderate fall in arterial B.P. and a rise in peripheral vascular resistance (3, 13, 24, 32, 33). A similar picture is seen during experimental hypothermia (28). The hemodynamic picture that accompanies severe poisoning by hypnotic drugs may be caused by one or more of the following factors: 1) hypovolemia (37), 2) inadequate tone of capacitance vessels (11), 3) reduced myocardial force (6, 9), 4) impaired regulation of resistance vessels (1). In the literature the clinical picture is often referred to as shock when marked hypotension is present (8, 32, 33). It is a matter of definition whether this term is justified or not. It is generally considered that the essential disturbance in the syndrome of shock is insufficient peripheral blood flow and tissue hypoxia, which is not necessarily present in cases with hypnotic drug poisoning accompanied by hypothermia and reduced metabolic demand.

Infusion of NE may be expected to be unfavourable when the arterial hypotension is mainly caused by hypovolemia (25). As judged by the present findings of a relatively normal central venous pressure, hypovolemia is not likely to be the main factor. The present study of patients with hypotension because of poisoning by hypnotic drugs shows that the pressor effect of NE is largely accomplished by an increase of C.O. without significant change of peripheral vascular resistance. In case 7 there was a decrease in HR and no increase in C.O., therefore the infusion of NE was discontinued. However, the patient's condition deteriorated, with no measurable B.P. about one hour after the study. Because of this, NE was infused again and the patient subsequently

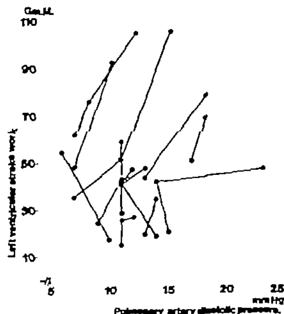


Fig. 4. Left ventricular work in relation to pulmonary artery diastolic pressure. Symbols as in Fig. 1.

recovered. Available information regarding the effects of drug, body temperature, CVP or blood gas disturbances did not provide any simple correlation to the variations in NE response. It has been demonstrated that NE increases B.P. and usually C.O. in patients with hypotension because of myocardial infarction (18, 22) and also in a mixed material of 19 patients with hypotension (1 drug poisoning) and in whom infectious diseases dominated (31). In these materials there was a significant increase in peripheral vascular resistance contrasting with the findings in the present study.

It is well known that infusion of NE to healthy subjects causes an increase in B.P., reflex bradycardia, unchanged or decreased C.O. with a marked increase in peripheral vascular resistance. This implies that the α-receptor stimulating effect is dominant in healthy subjects. However, in patients with hypotension because of hypnotic drug poisoning the β-receptor stimulating effect on the myocardium may be quite marked, as illustrated by the present results. The increase in left ventricular stroke work reflects the positive inotropic effect of NE. Also a reflex bradycardia is usually absent. This indicates that the normal B.P. regulation via the arterial baroreceptors is disturbed by the poisoning.

The pulmonary arterial diastolic pressure reflects left atrial pressure in the absence of increased pulmonary vascular resistance (30). Animal studies indicate no pulmonary vasopressor action of NE if flow and left atrial pressures are kept constant (15), but investigations in man are contradictory (5, 17). Pulmonary artery pressures were increased in some patients. The increase in pressure seen with increasing doses of NE may result from an increase of C.O., although it is generally thought that pulmonary vascular pressures remain relatively constant over a wide range of blood flows. The increase in pulmonary artery diastolic pressure probably reflects an increase in left atrial pressure (21) and could be caused by an increase of venous tone resulting in a shift of blood to the central circulation.

Folkow et al. (14) demonstrated a constrictor effect of NE on the capacitance vessels. Reduced venous pooling and increased return because of venoconstriction induced by NE may be contributory factors to the suggested dominant cardiac effect with increased C.O. (19). However, long-term treatment with NE and allied vasopressor substances brings a reduction of the plasma volume (26). The reason for this may be a constriction of postcapillary sphincters with increase in capillary hydrostatic pressure. Isoproterenol, a pure β-receptor stimulating agent given to patients with drug poisoning and hypotension, results in tachycardia, increased C.O., lowered mean arterial and diastolic pressures, while the systolic pressure is relatively unchanged (33). Since administration of a pure β-receptor stimulating agent reduces mean arterial B.P., the effect of NE must be a balance between the α- and β-receptor stimulating properties. Thus both the central β- and the peripheral α-receptor stimulating effects contribute to the B.P. rise. However, the balance between the α- and β-receptor effects varied and it is reasonable to assume that the B.P. rise resulted primarily from a peripheral effect in cases with relatively less marked increase of C.O.

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ON CORTICOSTEROID AND AZATHIOPRINE TREATMENT OF GLOMERULAR RENAL DISEASES

A Study of 90 Cases, Treated 1964–1969

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Abstract. The results after corticosteroid and azathioprine treatment of glomerular renal disease were assessed in a series of 90 patients. Forty-five (59%) of 76 patients treated solely with corticosteroids for at least one month showed an improvement, while 20 were unchanged and 11 deteriorated. Azathioprine was given to 37 patients, 31 of whom were treated with corticosteroids as well. In this group of patients, who were more severely ill than those treated only with corticosteroids, 12 (32%) improved. The hypertensive patients had a worse prognosis than the normotensive, but otherwise it was not possible from the clinical data and the light microscopy examination of renal biopsies to predict which of the patients would react favourably to the treatment. Without a real control series it is impossible to get a clear evaluation of such treatment, but the results in individual cases appeared so good that we feel it ethically impossible to refuse a badly ill patient a treatment which may be of benefit.

The use of immunosuppressive drugs in the treatment of patients with glomerulonephritis has been practised for some 20 years (6). The often successful treatment of renal transplant patients with corticosteroids and immunosuppressive drugs among others, intended to prevent and reduce rejection, has led to a general use of this sort of therapy in various forms of glomerular renal diseases (e.g. 1, 3, 4, 8, 9, 11, 12, 13, 14, 15, 17, 18, 19). Apart from favourable results in systemic lupus erythematosus the value of such treatment for glomerulonephritis seems to remain largely open to question, and there is difficulty in comparing different reported series.

SOME GENERAL REMARKS AS A BACKGROUND TO THE PATIENT MATERIAL AND TO THE TREATMENT AND METHODS USED

Considering the possibility or probability that the above mentioned medical therapy may be of value in glomerular renal disease, it is difficult or impossible for the clinician not to give such treatment in certain cases simply in order to obtain a reliable control material. On the other hand this therapy involves definite risks which affect the dosage and the indications for treatment. It is therefore not possible to judge to what extent the dosage used and the combinations of medicaments were optimal or adequate. This also applies to the time at which the treatment was given and its duration. The guiding principle for applied therapy has been the old maxim primum non nocere.

In this series medical therapy was limited to corticosteroids and/or azathioprine. Other presently relevant means of treatment, with, e.g. actinomycin C, cyclophosphamide, indomethacin, antihistamines and anticoagulants have not been employed.

It is well known that the mortality of children with acute glomerulonephritis has been reduced by 80–90% during the last two decades, and that this fact has been connected with the early initiation of antibiotic treatment for streptococcal infections of the pharynx and elsewhere. Nowadays cases of chronic glomerulonephritis are also being treated with antibiotics either in the event of, e.g., upper respiratory tract infections or as regular long-term therapy. The effects of immunosuppressive treatment, in themselves favourable, could conceivably be masked by increased sensitivity to infections which are not diagnosed or not compensated for by simultaneous antibiotic therapy. For these reasons it was felt that there was no justification for trying to obtain a control series through comparison with a series from previous years without immunosuppressive therapy. Thus the spontaneous course of the disease in the series discussed here cannot be determined.

Contemporaneously with the introduction of immunosuppressive therapy at this clinic an important change has taken place in the treatment of glomerulonephritic patients. Traditional methods of treatment, such as confinement to bed, rest, dieting and the restriction of salt supply have largely been abandoned. It is true that the value of such treatment had neither with certainty been proved nor disproved. It is perhaps worth pointing out that a form of therapy of possibly uncertain value has been rejected in favour of a modern therapy with, to date, largely unproven effects.

Table I. *Number of patients improved, unchanged or deteriorated, grouped with regard to the duration of disease before treatment*

Duration of disease before treatment (mo.)	No. of pats.	
	Improved	Unchanged or deteriorated
<2	17	4
3–12	16	11
13–36	7	4
>36	5	12
Total	45	31

As will appear from the following, certain clinical and laboratory criteria have been applied when making general assessments of the indications for treatment and of the effects of the therapy. It would, of course, have been desirable to have other criteria as well. Specimens obtained from percutaneous renal biopsy were examined by light microscopy. Electron microscopic examination has unfortunately not been carried out. Immunofluorescence microscopy was only possible in isolated cases, and findings from it are therefore not included in the evaluation of the therapy and its effects in this series of patients.

THE SERIES OF PATIENTS

The material comprised those 90 patients who during a 5-year period (1964–69) received treatment at this clinic and who fulfilled the requirements of being diagnosed by renal biopsy and treated with corticosteroids and or azathioprine for at least one month. Of the 90 patients ... were men and 30 women. The mean age was 34.3 years (range 9–70). Only 5 patients were younger than 15 years.

For analysis the material was divided into two groups. The first group contained 76 of the 90 patients treated only with corticosteroids. The second group comprised 37 patients receiving azathioprine treatment. Six of these patients were treated only with azathioprine, while in 31 this treatment was combined with corticosteroid treatment. Twenty-three patients were members of both groups and were assessed on two occasions, firstly after a period with only corticosteroid treatment and secondly after a period during which azathioprine was also given.

METHODS

The diagnosis of glomerular renal disease was made on the basis of the results from clinical examinations and from biopsies.

The clinical course of the disease was judged after at least one month's treatment in accordance with the criteria in the following five groups: 1) *Complete remission.* All examination results normal for six months. 2) *Incomplete remission.* The condition of the disease improved, but examination results not fully normalized, the following limits being set: quantitative urine protein of max. 2‰ for nephrotic patients and max. 0.5‰ for other patients; haematuria in quantitative sediment according to Addis of max. 10 mill. red blood cells/12 hours or in semiquantitative sediment of max. 15 red blood cells/microscopic field (320) and a sedimentation rate of max. 20 mm/1 hour. Other conditions were that serum electrophoresis was normal or almost normal and that endogenous creatinine clearance, if this was initially lowered, had normalized or improved by at least 20 ml/min during the time of treatment. 3) *Partial improvement.* Improvement of the condition whereby at least two but not all of the criteria under point 2 were fulfilled. 4) *Unaltered condition.* Examination results essentially unaltered. 5) *Deteriorated.* One or more of the examination results showed deterioration.

Specimens for microscopical examination were obtained by percutaneous needle biopsy (10). No serious complications were seen. The biopsy material was fixed in Bouin's solution and sections were routinely stained with Weigert haematoxylin and eosin, by the periodic acid/Schiff technique, according to Gleson, and by Gridley's silver reticulum method, sometimes counterstained with Kernechtrot.

The renal changes were classified according to conventional principles. For the diagnosis of latent glomerulonephritis the usual criteria were applied (7). The cases classified as minimal disease showed clinically a nephrotic syndrome and anatomically normal or almost normal glomeruli as seen by light microscope.

RESULTS

Corticosteroid therapy

Treatment was not commenced on the basis of a coordinated scheme but was judged from case to case. Account was taken partly of the clinical activity from the disease measured from the degree of proteinuria, reduction in renal function, changes in serum electrophoresis and rise in ESR, among others, and partly of the renal biopsy. The dosage of corticosteroids varied. An initial dose of 40 mg prednisolone/day or more was given to 16 patients, 20–35 mg to 34 and less than 20 mg to 26. The initial dosage was maintained unchanged for 1–4 weeks, after which it was successively reduced. The average period of treatment for the total material of 76 patients was 12 months (range 1–48).

If the total material of 76 corticosteroid-treated patients was considered independently of the diagnosis, 8 (11%) showed complete remission, 20 (26%) incomplete remission and 17 (2.%) partial improvement. The condition was unaltered in 20 (26%) and had deteriorated in 11 (14%) of the cases. Thus an improvement could be noted in

Table II. *Clinical findings in relation to the renal diagnoses in 76 patients treated with corticosteroids*

Type of renal disease	No. of pats.	No. of pats. with: Anamnesis of acute renal disease	Preceding infections	Increased antistreptolysin titre	Nephrotic syndrome	Reduced renal function	Arterial hypertension
Acute glomerulonephritis	9	9	7	5	2	4	5
Proliferative	7	7	5	4	1	3	4
Exudative	1	1	1	0	0	1	1
Mixed type	*1*	*1*	*1*	*1*	*1*	*0*	*0*
Progressive acute glomerulonephritis	4	4	2	2	3	4	3
Chronic latent glomerulonephritis	11	7	7	4	3	3	0
Chronic glomerulonephritis	35	10	13	18	18	13	16
Membraneous	9	1	2	3	8	4	3
Proliferative	9	5	5	8	1	3	3
Lobular	4	2	1	1	1	3	3
Mixed type	13	2	5	6	8	3	7
Focal glomerulonephritis	5	3	3	2	1	1	1
Minimal disease	3	1	0	0	3	1	0
Interstitial nephritis	1	0	0	0	0	1	1
Collagen disease	8	0	0	3	2	5	4
Total	76	34	32	34	32	32	30

altogether 45 cases, constituting 59% of all the patients treated.

In order to assess the effects of the dose administered the material was divided into groups which were initially given less than 20, 20–35 and 40 mg or more prednisolone/day respectively. The results of the treatment were not significantly different in the different dosage groups whether the material was treated as a whole or whether it was subdivided into groups on the basis of the patho-anatomical pictures.

With regard also to the length of the treatment there was no difference between the improved group as compared with the group of unaltered or deteriorated.

Table I shows, respectively the number of improved and unaltered or deteriorated conditions among the 76 cases treated with corticosteroids in relation to the duration of the disease prior to the commencement of the treatment. Seventeen of the 21 patients with a duration of disease of 2 months or less improved, while only 5 of the 17 with a duration of longer than 36 months showed any improvement. The difference between these figures is significant ($p < 0.005$). There was no significant difference between the other groups. It is interesting to note, however that in the groups with a duration of disease prior to treatment of up to 36 months an improvement could still be obtained in over half the cases.

Acute onset was present in 34 (45%) of the 76 patients (Table II) in all the 13 cases (100%) with acute glomerulonephritis at renal biopsy but only in 10 (29%) of 35 cases with chronic glomerulonephritis. The difference was significant ($p < 0.001$). There was an acute onset in 7 (64%) of 11 patients with latent glomerulonephritis but in none of the 8 with collagenous disease. There was no difference in the frequencies of reduced renal function between the groups which had and did not have anamnesis of acute renal disease.

Sore throat or other infections prior to the onset in question were found in 32 (42%) of the 76 patients and were most common in the groups with acute and latent glomerulonephritis (69% and 64% respectively). This was significantly different from the group with collagenous disease, in which no preceding infections were noted, but

Table III. *Clinical findings in relation to result in 76 patients treated with corticosteroids*

Result	No. of pats.	No. of pats with: Anamnesis of acute renal disease	Preceding infections	Increased antistreptolysin titre	Nephrotic syndrome	Reduced renal function	Arterial hypertension
Complete remission	8	5	5	3	3	0	1
Incomplete remission	20	12	9	8	8	6	4
Partial improvement	17	5	6	8	9	10	7
Unaltered	20	10	8	11	7	6	7
Deteriorated	11	2	4	4	5	10	11
Total	76	34	32	34	32	32	30

not significantly different from the corresponding frequencies in the other groups of patients. The 32 patients with anamnesis of infection showed no significant difference with regard to frequency of reduced renal function and results of treatment from the other 44 patients (Table III)

Increased antistreptolysin titre was found in 34 (45%) of the 76 patients. Their distribution within the different diagnosis groups was quite even. This was also the case within the different groups of the clinical course of the disease.

Reduction in renal function as measured by endogenous creatinine clearance was recorded in 32 (42%) of the 76 patients. Of these 32 patients ıe renal function improved in 11 during the of observation. Of the 13 patients with biopsy changes of the type acute glomerulo- ephritis, renal function was reduced in 8 (62%) of whom 5 improved. Of the 35 patients with chronic glomerulonephritis renal function was reduced in 13 (37%) of whom 3 improved. There are no statistically significant differences either between these frequencies or between the corresponding frequencies of the other diagnosis groups.

Arterial hypertension with BP of 160/100 or above was found in a total of 30 (39%) of the 76 patients treated with corticosteroids. There was no significant difference in the frequency of nephrosis in the hypertensive patients as compared to the normotensive. In the group with latent glomerulonephritis there were no hypertensive cases, this being significantly different from the groups with acute glomerulonephritis, chronic glomerulonephritis and collagenous disease. Of the 13 cases with acute glomerulonephritis 8 (62%) had hypertension, while in the group with chronic glomerulonephritis 16 (46%) out of 35 patients had hypertension.

There was a relationship between BP and renal function. Of 30 patients with hypertension renal function was reduced in 18 (60%) while of the 46 patients without hypertension it was reduced in 14 (30%) The difference was significant ($p < 0.025$) During the corticosteroid treatment 33 (72%) of the 46 normotensive patients, but only 12 (40%) of the 30 hypertensive, improved (Table IV) Here, too the difference was significant ($p < 0.025$)

Acute glomerulonephritis occurred patho-anatomically in 9 cases in all, of which 7 with mainly proliferative, 1 mainly exudative and 1 mixed changes. In all these patients there was an acute onset, and in 5 cases biopsy was performed within

Table IV *Arterial pressure and outcome in 76 patients treated with corticosteroids*

Arterial BP	Complete remission	Incomplete remission	Partial improvement	Unaltered	Deteriorated	Total
>160/100	1	4	7	7	11	20
<160/001	7	16	10	13	0	46
Total	8	20	17	20	11	76

Table V *Patho-anatomical diagnoses and outcome in 76 patients treated with corticosteroids*

Type of renal disease	Complete remission	Incomplete remission	Partial improvement	Unaltered	Deteriorated	Total
Acute glomerulonephritis	1	4	1	3	0	9
Proliferative	0	4	1	2	0	7
Exudative	0	0	0	1	0	1
Mixed type	1	0	0	0	0	1
Progressive acute glomerulonephritis	0	1	2	0	1	4
Chronic latent glomerulonephritis	2	3	3	3	0	11
Chronic glomerulonephritis	3	8	7	10	7	35
Membranous	1	3	2	1	2	9
Proliferative	1	4	2	2	0	9
Lobular	0	0	0	1	3	4
Mixed type	1	1	3	6	2	13
Focal glomerulonephritis	0	2	0	3	0	5
Minimal disease	1	1	1	0	0	3
Interstitial nephritis	0	0	0	0	1	1
Collagen disease	1	1	3	1	2	8
Total	8	20	17	20	11	76

2 months of the onset. In 3 cases, however the acute onset occurred more than 6 months prior to the biopsy In spite of this long anamnesis, which meant that from the clinical point of view these cases could virtually be regarded as chronic, only acute changes were found at renal biopsy with no signs of a chronic process. In 2 of the 9 cases there was a nephrotic syndrome. Renal function was reduced in 4 patients and during the period of observation it improved in 2 of them.

During the period of observation 6 of the 9 cases improved, while the condition in 3 cases was judged to be unaltered (Table V).

Progressive acute glomerulonephritis was present in 4 cases. All had an acute onset and all showed reduced renal function. During the treatment period, 3 of the patients improved and 1 deteriorated.

Chronic latent glomerulonephritis was present in 11 patients. Seven of them had an anamnesis of acute renal disease and the average time of the disease prior to the biopsy amounted to fully one year Three of the patients initially had reduced renal function, which in 2 cases improved during the period of observation. It should also be noted that none of the patients in this group showed any rise in BP

The course of the disease in this group was favourable in that 8 patients showed an improvement during the period of observation, while the remaining 3 showed no change.

Chronic glomerulonephritis was diagnosed in 35 (46%) of the 76 patients treated with corticosteroids. Patho-anatomically the glomerulonephritis was membranous in 9 proliferative in 9 and lobular in 4 cases, while in 13 cases the changes were of mixed type. Ten patients had an anamnesis of acute renal disease of whom 5 at renal biopsy were found to have proliferative changes. Eighteen of the patients had a nephrotic syndrome, the biopsy being membranous and mixed in 8 cases each, and proliferative and lobular in 1 case each. Renal function was reduced in 13 patients in all, of whom only 3 improved. During the period of observation 18 (51%) of the 35 cases improved, in 10 (29%) the condition was unaltered and in 7 (20%) deteriorated.

Focal glomerulonephritis was observed in 5 patients. Two of these improved during the period

Table VI. *Clinical findings in relation to renal diagnoses in 37 patients treated with azathioprine*

Type of renal disease	No. of pats.	No. of pats. with: Anamnesis of acute renal disease	Preceding infections	Increased antistrepto-lysin titre	Nephrotic syndrome	Reduced renal function	Arterial hypertension
Progressive acute glomerulonephritis	3	3	1	1	2	3	3
Chronic latent glomerulonephritis	2	1	1	0	1	1	0
Chronic glomerulonephritis	22	5	11	12	11	13	11
Membranous	4	1	2	2	3	2	1
Proliferative	5	2	4	2	1	2	1
Mixed type	13	2	5	8	7	9	9
Minimal disease	1	0	0	0	1	1	0
Interstitial nephritis	2	0	0	0	1	2	2
Collagen disease	6	0	0	1	3	4	5
Renal amyloidosis	1	0	0	0	1	1	0
Total	37	9	13	15	20	25	21

of observation. The length of anamnesis in these 2 cases was 1 and 2 months, respectively being longer in the remaining 3 cases with an unaltered condition. One of these patients had a nephrotic syndrome

Renal changes of type *minimal lesions* were in 3 cases. All of them had an anamnesis nephrotic syndrome for several years. One pa who had a complete remission, had a 2 year anamnesis at the time when the corticosteroid treatment was commenced. This continued for years, during which time the condition normalized. Since the treatment was broken off the period of observation has continued for a further years without relapse. The remainder had frequent relapses despite the corticosteroid treatment.

Interstitial nephritis was seen in one patient in whom there was some suspicion of hereditary nephropathy Corticosteroid treatment was attempted, since the patient appeared clinically to have glomerulonephritis with typical urinary sediment findings and a nephrotic syndrome. During a later period this patient was also treated with azathioprine, but renal function showed increasing deterioration and the patient later received kidney transplant.

Collagenous disease This group comprised 8 patients of whom 3 had a classic systemic lupus erythematosus. Two of these 3 patients were the ones judged as complete and incomplete remission, respectively In the remaining 5 patients the renal disease was a component of inter alia, Wegener s granulomatosis and periarteritis nodosa.

Azathioprine therapy

Treatment with azathioprine (Imurel®) was given to 37 patients in all. The dose was initially 1.5–3 mg/kg b.wt. and 24 hours. The average period of treatment was 12 months (range 1–36) Assessment and control of these patients were carried out in the same way as for the corticosteroid-treated patients except that the number of white blood cells was checked, first daily and later at up to 3-week intervals. If the WBC dropped below 3 000/mm³ the treatment was broken off for some days and then recommenced, usually with the same dosage In addition serum bilirubin and transaminases (GPT GOT and LDH) were checked at intervals of 1–3 months.

The indications for commencing azathioprine treatment were much more strictly defined than those for the corticosteroid treatment. Thus azathioprine treatment was commenced partly in cases when corticosteroids alone produced no or only slight effects, and partly when the clinical and histological examination showed such a serious condition for the patient that it was considered necessary to employ all available thera-

Table VII. *Clinical findings in relation to result in 37 patients treated with azathioprine*

Result	No. of pats.	No. of pats. with: Anamnesis of acute renal disease	Preceding infections	Increased antistreptolysin titre	Nephrotic syndrome	Reduced renal function	Arterial hypertension
Complete remission	1	0	0	0	0	0	1
Incomplete remission	4	1	1	0	3	1	1
Partial improvement	7	1	1	2	4	3	5
Unaltered	13	4	5	8	8	9	5
Deteriorated	12	3	6	5	5	12	9
Total	37	9	13	15	20	25	21

peutic resources. Thus, in comparison to those treated with corticosteroids, the azathioprine-treated patients had much more serious renal conditions. Twenty-five (68 %) of the 37 patients treated with azathioprine had renal insufficiency (Table VI) as opposed to 32 (42 %) of the 76 patients treated with corticosteroids. The difference between these frequencies was significant ($p < 0.025$).

With regard to other data from anamnesis and laboratory investigations there were no significant differences between the two treatment groups.

If the total azathioprine material of 37 patients was considered independently of the diagnosis, 1 was found to have a complete remission, 4 incomplete remission and 7 partial improvement, the condition was unaltered in 13 cases and had deteriorated in 12 (Table VII). In all, then, an improvement was noted in 12 (32 %) of the total number of patients treated. In terms of results, the patients who received combined azathioprine and corticosteroid treatment showed no difference from those who received azathioprine treatment alone, there being, however, only 6 such patients.

The distribution of the 37 patients treated with azathioprine among the patho-anatomical groups, and their grouping with regard to the clinical course of the disease, will be seen in Table VIII. Twenty-two (59 %) of the 37 patients had chronic glomerulonephritis, while other diagnoses occurred in only a few cases. There was only 1 complete remission in a case with periarteritis nodosa, the observation period under continued azathioprine treatment being now $2^1/_2$ years. Incomplete remission was recorded in 4 patients, of whom 3 had chronic glomerulonephritis without reduc-

Table VIII. *Patho-anatomical diagnoses and outcome in 37 patients treated with azathioprine*

Type of renal disease	Complete remission	Incomplete remission	Partial improvement	Unaltered	Deteriorated	Total
Progressive acute glomerulonephritis	0	0	1	1	1	3
Chronic latent glomerulonephritis	0	0	0	2	0	2
Chronic glomerulonephritis	0	3	3	7	9	22
Membranous	0	0	0	3	1	4
Proliferative	0	1	1	2	1	3
Mixed type	0	2	2	2	7	13
Minimal disease	0	1	0	0	0	1
Interstitial nephritis	0	0	0	1	1	2
Collagen disease	1	0	3	2	0	6
Renal amyloidosis	0	0	0	0	1	1
Total	1	4	7	13	12	37

tion in renal function. In 1 case with renal amyloidosis and a nephrotic syndrome on the bases of spondylitis anchylopoëtica, azathioprine treatment was attempted without effect.

Complications

During treatment with *corticosteroids* 7 patients revealed psychic disturbances—in 2 cases so grave that care in a psychiatric department was necessary and in the remaining 5 cases in the form of slight depression.

Eye complications occurred in 3 patients, namely a posterior polar cataract in one, retinal oedema with bleeding in one and a papillary oedema in one. This last complication appeared during a period when the corticosteroid dosage was reduced and regressed when the dose was increased again. In 3 patients venous thrombosis of the leg occurred simultaneously with the corticosteroid treatment. One of these patients contracted lung emboly which led to exitus. One uraemic patient (serum creatinine 13 mg%) developed acute, fatal gastric bleeding.

The *azathioprine* treatment caused temporary leucopenia in 10 cases, necessitating temporary breaking-off of the treatment. A 25-year-old woman with progressive acute glomerulonephritis with anuria, which, apart from dialysis, was treated with a combination of corticosteroids and oprine for one month, developed fulminant, mycotic septicaemia. There were no other serious infectious complications.

In 3 cases there was an increased tendency to anaemia, all these patients being, however uraemic. In one patient the azathioprine treatment was broken off because of pronounced nausea.

DISCUSSION

The number of improved patients amounted to 45 of 76 (59%) treated with corticosteroids and 12 of 37 (32%) treated with azathioprine. The pathoanatomical examination at renal biopsy gave no sure indications as to which patients would react favourably to the treatment. It must be noted, however that some diagnosis groups comprised only a few patients.

Where hypertension occurred in this material, it proved to be prognostically significant. Of the 76 corticosteroid-treated patients the frequency of those improved was significantly higher in the normotensive (72%) than in the hypertensive patients (40%). In this group there was also a relationship between BP and renal function. Hypertensive patients had reduced renal function in 60% normotensive patients only in 30% The frequency of improved patients, however was not significantly different between those who had normal renal function (66%) and those who had lowered renal function (50%) These figures indicate that reduced renal function is not in itself an obstacle to favourable development, but that the occurrence of hypertension is prognostically a bad sign. But the question concerning the extent to which this worse prognosis is conditioned by a more serious underlying renal disease in the hypertensive cases, or whether it is conditioned by the hypertension per se remains unanswered. The risk that a glomerular renal disease may develop a secondary nephrosclerosis should be respected. The BP of these patients should be carefully watched and treated early if it shows a tendency to rise.

With the type of immunosuppressive treatment used many patients showed a clear improvement of the disease. In the case of renal diseases of this kind there is a certain frequency of spontaneous remissions, which may occur even after several years of illness (2) The question is then to what extent the observed improvement is due to or independent of the treatment. For some of our patients we had been able to follow their renal disease and record its activity etc. for relatively long periods. If then there was a clear improvement in immediate conjunction with the treatment, this could with reasonable probability be interpreted as a therapeutic effect. In order to obtain a reliable evaluation of immunosuppressive treatment, the series of patients must comprise matched control cases who are not given treatment (5 8) but it has been discussed whether such a procedure is ethically defensible (16).

To sum up we think that it is at present justified, irrespective of the clinical and pathoanatomical specification of an active glomerulonephritis, to attempt treatment with corticosteroids and/or azathioprine despite the risks involved.

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INSULIN SECRETION AFTER TOLBUTAMIDE AND AFTER SECRETIN IN PATIENTS WITH PANCREATIC DISEASES

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Abstract. In 20 patients with different pancreatic disorders the endocrine and the exocrine pancreatic function has been studied. The endocrine function was characterized by the glucose tolerance and by the changes in blood glucose and serum insulin concentration after tolbutamide and after secretin given intravenously. The exocrine pancreatic function was characterized by the concentration of amylase and lipase in the duodenal contents after ingestion of a meal and by the fecal fat excretion. The glucose tolerance was reduced in nine patients; it was not correlated to the exocrine pancreatic function. The insulin secretion following tolbutamide was significantly reduced, most pronouncedly in patients with severe decrease of the exocrine pancreatic function. The insulin secretion following secretin was significantly reduced too. Patients with severe reduction of the exocrine pancreatic function failed to react with insulin secretion after secretin, although the same patients reacted with insulin secretion after tolbutamide. It is concluded that the effect of secretin on insulin secretion might be a result of the secretin-induced change of the pH in the pancreatic tissue.

The diabetes mellitus attending chronic pancreatitis is probably a result of the inflammatory reaction with hyalinization and degeneration of the islet tissue. Genetically determined dysfunction of the β-cells seems to play a subordinate role.

In normal subjects the secretion of insulin is stimulated by tolbutamide (4) as well as by secretin (1). In patients with diabetes mellitus a discrepancy between the effect of tolbutamide and secretin on insulin secretion has been observed (2, 3). The aim of the present study was to investigate the secretion of insulin following stimulation by secretin and tolbutamide in patients with pancreatic diseases.

MATERIAL

The material comprised 20 patients with different pancreatic disorders. The composition of the material is shown in Table I. Pancreatic cancer, fibrosis or cysts were in all cases verified at operation. Calcifications were seen at X-ray.

One of the patients (no. 16) was slightly overweight according to Natvig (13). The other patients were normal or underweight.

In all patients the renal function, estimated as the concentration of creatinine in serum, was normal; so was the blood pressure. Two of the patients (nos. 10 and 11) exhibited slightly elevated concentrations of alkaline phosphatase in the serum and hypergammaglobulinaemia. Furthermore the values of bilirubin concentration, alkaline phosphatases and GOT concentrations in serum as well as prothrombin proconvertin concentration in plasma and serum protein electrophoresis were normal. Liver biopsy was performed in seven patients. Cirrhosis was observed in one, steatosis in three, while [illegible] normal finding was observed in three patients.

All patients were ambulatory at the time of the study and received a diet containing more than 150 g carbohydrates/day.

Twenty-eight subjects, members of the hospital staff or patients with minor diseases as earlier described (4), served as controls.

METHODS

In each patient the following investigations were performed: 1) glucose tolerance test, 2) exocrine pancreatic function test, 3) fecal fat, 4) [illegible] tolbutamide test, and 5) i. [illegible] secretin test.

The glucose tolerance was studied in 12 patients by means of an i.v. glucose tolerance test according to Lundbæk (10), the disappearance rate of glucose (K) being calculated as

$$K = \frac{\ln 2}{t} \cdot 100$$

where t was obtained by the graphical method from the curve of glucose concentrations plotted semilogarithmically against time. In the other eight patients the glucose concentration in blood was followed for 2 h after oral administration of 1 g glucose/kg b.wt. If the K value was < 0.90 or if the blood level of glucose 2 h after oral administration of glucose was > 150 mg/100 ml, the glucose tolerance was considered diabetic.

Table I. *The material, data and findings*

Pat. no.	Age (y.)	Sex	Clinical data	B.wt. (% of ideal wt.)	Fasting blood sugar concentration (mg/100 ml)	Glucose tolerance[a]	Fecal fat (g/24 h)	Conc. of amylase in intest. contents after meal[b] (kU/l)
1	57	♀	Pancreatic calcifications, exocrine insufficiency	102	82	ND	106	8
2	48	♂	Pancreatic calcifications, chronic alcoholism	88	83	ND	80	12
3	53	♀	Pancreatic fibrosis, exocrine insufficiency Billroth I resection	83	313	D	65	46
4	76	♀	Pancreatic cancer gastro-enteroanasto-mosis	73	70	ND	60	1
5	64	♂	Pancreatic fibrosis, exocrine insufficiency Billroth I resection	100	108	D	58	4
6	50	♀	Relapsing pancreatitis, exocrine insufficiency	79	58	ND	50	57
7	49	♂	Pancreatic fibrosis, pancreatic cysts, pancreatic calcifications	79	83	ND	39	—
8	61	♂	Pancreatic calcifications, exocrine insufficiency	90	63	ND	30	18
9	40	♂	Pancreatic cancer exocrine insufficiency	68	72	D	30	13
10	52	♂	Relapsing pancreatitis, exocrine insufficiency	69	86	D	29	12
11	44	♂	Pancreatic fibrosis, pancreatic cysts	86	88	ND	16	—
12	42	♂	Relapsing pancreatitis, chronic alcoholism	98	188	D	9	71
13	64	♂	Pancreatic fibrosis	107	107	ND	9	14
14	18	♂	Relapsing pancreatitis, hyperparathyroidism	105	85	ND	7	65
	59	♂	Relapsing pancreatitis, chronic alcoholism	81	60	D	5	24
	46	♂	Relapsing pancreatitis, chronic alcoholism	117	114	D	3	34
17	48	♀	Pancreatic fibrosis, pancreatic cysts	81	111	D	3	85
18	59	♀	Relapsing pancreatitis	72	79	ND	3	101
19	46	♀	Pancreatic fibrosis	91	85	D	3	216
20	59	♂	Pancreatic fibrosis, chronic alcoholism	84	89	ND	1	40

ND = non-diabetic, D = diabetic.
[b] Normal values (m ± 2s), 139–780 kU/l.
Normal values (m ± 2s), 136–687 kU/l.
[a] Maximal increase in serum insulin concentration after stimulation.

The exocrine pancreatic function test was performed as described by Worning and Müllertz (18), the concentration of amylase (and lipase) being measured in the duodenal contents 80 min after ingestion of a fluid standard meal.

The fecal fat was measured on pooled feces from at least 3 subsequent days.

The tolbutamide and the secretin tests were performed on different days in the morning after 12–14 h fast. Blood samples were taken from a peripheral vein without using a tourniquet. The blood glucose and the serum insulin concentrations were estimated before and after i.v. injection of 1 g sodium tolbutamide. Samples were taken 4, 5, 10, 20, 30 and 60 min after the injection. Blood glucose and serum insulin were in the same way estimated before and after i.v. injection of highly purified secretin (Gastro-intestinal Hormones, Stockholm, Sweden), 75 clinical units. The intervals after the injection were here 1, 2, 3, 4, 6, 8, 10, 15, 20 and 30 min.

Glucose concentration was determined in capillary blood using Hagedorn-Norman Jensen method or in plasma using the glucose oxidase method. The two methods gave identical values.

Conc. of lipase in intest. contents after meal[c] (IU/l)	Δ serum insulin[d] after Tolbutamide (μU/ml)	Secretin (μU/ml)
0	15	0
—	8	9
1	6	16
1	27	—
4	6	—
3	87	24
—	11	6
2	41	14
1	34	16
8	26	5
—	8	12
—	23	—
—	—	54
15	63	29
21	48	126
63	48	123
—	6	7
—	117	97
—	34	29
—	47	99

Serum insulin concentration was determined immunologically by modification of Hales' and Randle method (7) using ^{125}I-labelled pork insulin. Duplicates were run. The reproducibility of the glucose and the insulin assay from day to day were, expressed as coefficient of variation, 2.9 and 9.4% respectively

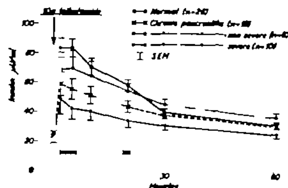


Fig. 1. Serum concentration of insulin (mean ± S.E.M.) before and after i.v. injection of 1.0 g tolbutamide in normal subjects and patients with pancreatic disease. Student's *t*-test between normal subjects and the whole group of patients: $-p < 0.05$ $-p > 0.02$, $-p < 0.01$

RESULTS

The glucose tolerance was reduced in nine patients; only two had frank diabetes. The glucose tolerance was not correlated to the exocrine pancreatic function irrespective of whether this was measured on the basis of the fecal fat excretion, the concentration of amylase or the concentration of lipase in the intestinal contents (Table I)

The exocrine pancreatic function varied considerably in the patients studied (Table I). Half of the patients presented marked steatorrhea and severe reduction of the concentration of lipase in the intestinal contents. In the remainder the fecal fat excretion was normal or slightly increased.

Consequently the material was subdivided into two groups, one *including patients with* severe reduction of the exocrine pancreatic function (nos. 1–10) and the other including patients with a normal or a slightly reduced exocrine pancreatic function (nos. 11–20).

The concentration of amylase in the intestinal contents was poorly correlated to the concentration of lipase and to the degree of pancreatic insufficiency (steatorrhea) (Table I)

The concentration of insulin in serum increased in all patients following tolbutamide (Table I). On an average, the increase was significantly smaller in the patients taken as one group than in the group of normal subjects. The insulin concentration was at any time higher in the group of patients with slight pancreatic reduction than in those with severe reduction (Fig. 1). However the maximal increase in serum insulin concentration following tolbutamide was only weakly correlated to the exocrine pancreatic function given

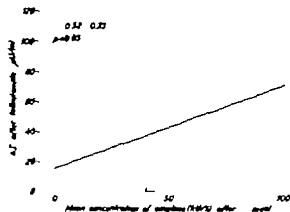


Fig 2. Correlation between exocrine and endocrine pancreatic function. ΔI = maximal increase of serum insulin concentration after i.v. tolbutamide.

as concentration of amylase in the intestinal contents (Fig. 2) or as fecal fat excretion (Fig. 3)

The decrease in concentration of glucose in blood following tolbutamide was on an average greater and more prolonged in the patients than in the normal subjects. Thus the blood concentration of glucose in patients with severe pancreatic reduction was significantly lower than in normal subjects 60 min after the injection (Fig. 4)

The increase in the serum concentration of insulin following secretin was very varied (Table I). Patients with severe reduction of the exocrine

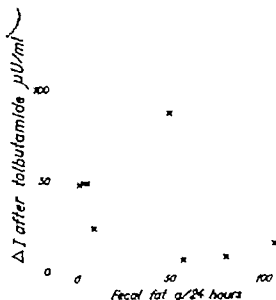


Fig. 3 Fecal fat excretion as compared to the maximal increase in serum insulin concentration after i.v. tolbutamide.

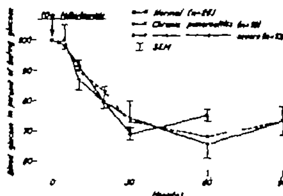


Fig. 4 Blood glucose concentration in % of fasting value (mean ± S.E.M.) after i.v. injection of 1.0 g tolbutamide in normal subjects, in the whole group of patients studied, and in the subgroup of patients with severe reduction of the exocrine pancreatic function. × indicates statistically significant difference between normal subjects and patients with severe reduction of the exocrine pancreas ($p < 0.05$).

pancreas presented no or only a slight increase in serum insulin concentration (Table I, Figs. 5 and 6) whereas patients with slight reduction of the exocrine pancreatic function exhibited a normal effect of secretin on serum insulin (Fig. 5). Fig. 6 illustrates the covariation between fecal fat excretion and maximal increase in serum insulin concentration after secretin in the patients studied. The correlation is rather poor but it is apparent that a normal increase in serum insulin concentration was observed only in patients with-

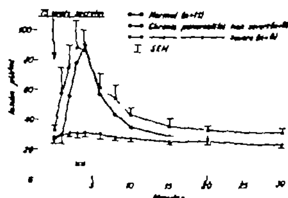


Fig 5 Serum concentration of insulin (mean ± S.E.M.) before and after injection of 75 clinical units highly purified secretin in normal subjects and in patients with non-severe or severe reduction of the exocrine pancreatic function. indicates a statistically significant difference between normal subjects and patients with severe reduction of the exocrine pancreas ($p < 0.01$)

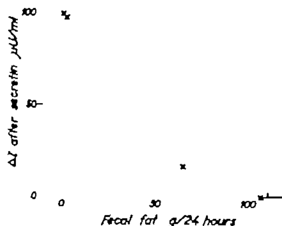


Fig. 4. Fecal fat excretion as compared to the maximal increase in serum insulin concentration after i. v. secretin.

out steatorrhea, whereas patients with marked steatorrhea presented an insignificant effect of secretin on the serum insulin concentration.

DISCUSSION

In the present material nearly half the patients presented a reduced glucose tolerance. Ohlsén (14) observed a similar abnormality in 20 out of 27 patients with chronic pancreatitis. The discrepancy is satisfactorily explained by the uneven composition of the two materials, as Ohlsén studied a more homogenous group of patients with rather servere chronic pancreatitis.

Endocrine pancreatic function, assessed by the insulin secretion following injection of tolbutamide proved to be significantly reduced in patients with chronic pancreatitis. Similarly Joffe et al. (9) demonstrated a reduced insulin response following i.v. tolbutamide in patients with chronic pancreatitis. Ohlsén (13) found the initial insulin response following i.v. injection of glucose to be reduced in the majority of patients with chronic pancreatitis, and also a reasonable correlation between, respectively, the endocrine and exocrine pancreatic function assessed by the insulin response following i.v. injection of glucose and the amylase secretion into the duodenum after secretin. In the present material the endocrine pancreatic function measured as insulin response after tolbutamide was only weakly correlated to the exocrine pancreatic function (Figs. and 3).

The great and prolonged fall in blood glucose concentration after tolbutamide in patients with severe reduction of the exocrine pancreatic function (Fig. 4) is remarkable considering the limited insulin secretion after tolbutamide in the same patients (Fig. 1). The explanation is probably the deficient glucagon secretion in patients with chronic pancreatitis (16).

It is striking that patients with severe exocrine pancreatic reduction exhibited no or only a very small increase in serum insulin concentration following secretin, although they showed an acceptable endocrine pancreatic function assessed by glucose tolerance and insulin secretion following tolbutamide. When the findings are compared to the results of secretin experiments on normalweight patients with stable diabetes mellitus, who gave a normal insulin response to secretin (2, 8) despite a considerably less marked insulin response to tolbutamide (3), the present investigations indicate that secretin acts upon the B cells of the islet tissue via factors which depend upon the exocrine pancreatic function. This accords with the observations of other workers who found that secretin-induced insulin secretion in rabbits could be eliminated despite preserved islet tissue function if the exocrine pancreatic tissue was destroyed by ligating the duct of Wirsung (5, 6). Raptis et al. (17) have also found a lacking insulin response following secretin in patients with chronic pancreatitis. The biochemical processes leading to insulin secretion following administration of secretin are still unknown. The secretin-induced shift in the pH of the pancreatic tissue secondary to the bicarbonate secretion might be responsible for the secretion of insulin either by an inhibition of the sodium pump of the B cells (12) or by promoting an influx of calcium ions into the B cells (11). According to this theory the reduced insulin secretion following secretin in patients with reduced exocrine pancreatic function is a consequence of the low exocrine secretion of bicarbonate in these patients.

It is well-known that the insulin secretion induced by an increase in glucose concentration in blood is potentiated when the glucose passes the gastrointestinal tract as compared to i.v. infusion

(15). It cannot be ruled out that this potentiation of insulin secretion may be due to functioning exocrine pancreatic tissue. Further studies are in progress in an endeavour to elucidate this question.

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INFECTIOUS MONONUCLEOSIS WITH THROMBOCYTOPENIC PURPURA

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Abstract. A case of infectious mononucleosis with long-standing thrombocytopenia, not responsive to corticosteroid therapy is presented. Rapid normalization of the platelet level was revealed by splenectomy. The patient is the fourth known case in which it has been necessary to perform splenectomy. A study made with ^{51}Cr-labelled homologous platelets showed a very short half-life, along with a high spleen/liver ratio of the activity. These examinations have not previously been performed in this condition.

As a rule infectious mononucleosis is a benign disorder most frequently found in young adults. Although it has been known for nearly 50 years that haemorrhagic manifestations may accompany the infection, no more than about 60 cases of infectious mononucleosis with thrombocytopenic purpura have been published. In general the thrombocytopenia has been of short duration. Most cases are spontaneously cured. If this is not the case, a good response has been achieved with corticosteroids. To the best of the author's knowledge it has been necessary to perform splenectomy in only 3 cases (8, 16, 26). This report deals with the fourth known splenectomized patient. The use of ^{51}Cr-labelled platelets enabled study of the half-life of the thrombocytes and the spleen/liver ratio, which had not previously been examined in this condition.

CASE REPORT

The patient is an 18-year-old female office clerk. Her parents, sister and brother are healthy. No bleeding manifestations or other haematological disorders are known in her relatives. Before the actual episode she had always been in good health. No haemorrhagic tendency or hypersensitivity reactions had occurred. Her menstrual bleedings have been normal.

At the beginning of Dec. 1970 the patient fell ill with a sore throat, but no fever. She did not take any drugs and the symptoms disappeared. One week later she observed an atraumatic bruise on her knee. On Dec. 30 her throat was sore again. She visited a local physician, who did not find any indication for antibiotic therapy. One week later, as the symptoms persisted, Eusaprim® (trimethoprim + sulfamethoxazole) was given for 2 weeks. The ESR, Hb, and WBC were normal. No differential counting of the white cells or platelet counting were carried out. The throat symptoms gradually disappeared, but the general condition was still impaired. On Feb. 13 1971 a number of bruises appeared on the arms and legs, together with widely distributed purpura on the legs. The bleeding time exceeded 30 min, the coagulation time was normal, and the platelet count 9 000/µl. The patient was admitted to hospital.

On admission the objective findings were as follows. The patient was short in stature, height 146 cm, weight 40 kg. Her general condition was fairly good, but she had a temperature of 38.6°C. There were purpura and skin bruises, as mentioned above, but no other bleeding. She was not icteric. The throat was red, and the tonsils were slightly enlarged. Neither lymph nodes nor the liver and spleen were palpable. The blood pressure was 125/80 mmHg, the pulse rate 108/min. Heart and lung auscultations were normal. The ESR was 22 mm/h, the Hb concentration 13.1 g%, and the WBC 5 400/µl. Differential counting showed 48% neutrophils (9.5% band forms), % eosinophils, 0.5% basophils, 4 % monocytes and 45% lymphocytes, of which one half were atypical, and led to a suspicion of infectious mononucleosis. The platelet count was 25 000/µl. The bleeding time was 9 min (Duke) and the Rumpel-Leede test was positive. The bone marrow specimen showed a very active picture. The granulocytopoiesis was hyperplastic, with a marked shift to the left but no blast cell infiltration. The megakaryocytes were numerous, but immature, with no visible platelet formation. The erythropoiesis was normoblastic and constituted 20% of the haematopoietic cells. The marrow finding excluded the possibility of acute leukaemia. The heterophil agglutinin titre (Paul-Bunnell) was 1:900, after adsorption on guinea-pig kidney 1:224, and after adsorption on beef erythrocytes 1:<14. Other laboratory examinations revealed normal serum bilirubin, blood glucose, liver and renal function tests and urinary sediment. The titres of antistreptolysin, antistaphylolysin, cold agglutinins and rheumatoid factor were normal. The Coomb's test was negative. Cryoprecipitation and nuclear antibodies were not demonstrated. LE cell

preparations were negative. The occurrence of platelet antibodies was studied repeatedly by the pplication of Coomb's technique, but never demonstrated. Antibodies to several viral infections were investigated, but all the titres were normal.

On admission to hospital profuse menstrual bleeding began t the time expected. In view of the simultaneous thrombocytopenic purpura, prednisone treatment was started. After therapy for 5 days the platelets had increased to 46 000, but again diminished to 17 000. By this time the purpura had disappeared, the menstrual bleeding had stopped, and confirmation was received that the patient was suffering from infectious mononucleosis. In consequence of earlier reports of the favourable prognosis of similar cases without treatment, the prednisone therapy was discontinued after period of 11 days, in particular as no response had become evident and the haemorrhagic manifestations had disappeared. The patient was then kept under observation for 10 weeks, but the platelet count did not exceed 20 000. The half-life of the platelets was now determined by means of ^{51}Cr-labelled homologous thrombocytes. The half-life was markedly shortened, $T\frac{1}{2}$ = 4–5 hours (normal value 3.5–4.5 days). The spleen/liver ratio at $T\frac{1}{2}$ was 4.4 (normal value 0.5–1.8) when the activity of the blood background was subtracted, and without subtraction 1.6 (normal value 0.5–0.9).

As the thrombocytopenia by this time had continued for 3 months, and it seemed probable that splenectomy would be the alternative treatment, second trial was undertaken with prednisone. The patient received initially 40 mg prednisone a day considered to be large dose in view of her smallness. She also developed moonface and acne in abundance; after 3 weeks the dose was reduced to 30 mg day which was administered for week. The therapy was then continued with smaller (20–10 mg day) and lasted for 5 weeks without response on the platelet level.

ough the patient had not suffered from any troublesome bleedings, it was considered hazardous to keep her on such low platelet level. At the beginning of Sept. 1971 splenectomy was performed after thrombocytopenia had continued for 6 months. The preoperative platelet count was 25 000. An immediate response was observed. On the first day after splenectomy the platelet count was 144 000, followed by 230 000, 372 000, 322 000, 454 000, and a maximum of 1 015 000 was attained on the sixth postoperative day. The platelet count then diminished, reaching a normal level within 2 weeks. At present, 4 months after splenectomy the platelet count is normal. The weight of the removed spleen was 70 g and showed normal histology.

The heterophil agglutinin titre, initially 1 : 900, increased and was 1 week later 1 : 1 800. After 4 weeks it had diminished to 1 : 224 and after 10 weeks to 1 : 28. The pathological lymphocytes disappeared within 2 weeks, subsequently the total number and the differential WBC have been normal. During the whole period of follow-up the Hb value has been normal.

The serum immunoglobulins were determined during the active phase of the disease and 1 month after splenectomy. No differences were discernible (IgG 12.0–12.2 g/l, IgA 1.8–1.7 g/l, IgM 1... 1.2 g/l).

Three months after presentation of the infectious mononucleosis, when the heterophil agglutinin titre had already become normal, but with the thrombocytopenia still persisting, an attempt was made to demonstrate circulating complexes of viral antigen-antibody and platelets by the application of a platelet aggregation test (19). However these complexes could not be demonstrated at that time. The test was not performed during the acute phase.

DISCUSSION

Thrombocytopenia in infectious mononucleosis is not a frequent finding. Pader and Grossman (17) found only 2 cases in a series of 300 patients. However with serial counting during the first week of symptoms, diminished platelet values were found in 5 out of 6 symptomfree patients (1), and in a series of 57 patients approximately 50% exhibited some degree of thrombocytopenia during the first 4 weeks of the disease (6). Bleeding manifestations, including epistaxis, haematuria, purpura and melaena, were early recognized in infectious mononucleosis. The first case of acute thrombocytopenic purpura, complicating proved infectious mononucleosis, was reported in 1942 (13). Since then reports have been published on about 60 similar cases. Detailed reviews of most cases up to 1963 have been published (7 21). In Scandinavia at least 5 confirmed cases have been noted, 3 in Denmark (2, 11 25) 1 in Sweden (12) and 1 in Norway (26).

The age of the patients concerned has ranged from 6 to 30 years, with a peak incidence in the late teens, which accords with the figures relating to the uncomplicated disease (4). In most cases the prognosis of the thrombocytopenia has been good. In general the symptoms have disappeared within 3 weeks and the platelet count has increased to normal level within 2 to 8 weeks. When treated with corticosteroids the symptomatic improvement and the return of the platelet count to normal have been more rapid (21). One long standing case (9 months) of moderate thrombocytopenia has been reported (22). A good initial response was noted with cortisone in the 18-year-old male patient, but the thrombocytopenia relapsed when the drug was dispensed with and the patient required steroids for approximately 6 months until a slow rise appeared in the number of platelets. Fatal cases of thrombocytopenia in infectious mononucleosis have also been reported, 1 case of cerebral haemorrhage (10) and 4 of splenic rupture (24).

The spleen has been palpable in about one half of the patients, but the enlargement has not borne any correlation with the severity of thrombocytopenia or the course of the disease. In 3 cases splenectomy has been performed. Dameshek and Grassi (8) observed a 22-year-old female with a past history of 2 years of bleeding manifestations before the onset of infectious mononucleosis, which aggravated the bleeding. A dramatic and sustained rise in the platelet count occurred on removal of the spleen (weight 324 g). However in this case it may be difficult to exclude coexistent chronic idiopathic thrombocytopenia. Ougier et al. (16) achieved a similar good response in an 8-year-old girl with corticosteroid-resistant thrombocytopenia which had persisted for 9 months. Recently Wetterhus (26) has reported on a 12-year-old girl with severe bleeding. Following unsuccessful prednisone therapy for 5 weeks, splenectomy was performed with a prompt rise in the platelet count.

The present case fulfilled the criteria for infectious mononucleosis. Previously no signs of bleeding disorders had been apparent, which excluded a coexistent haemorrhagic diathesis. However the clinical course was different from that of most cases reported earlier. First, the thrombocytopenia was long-standing, and secondly no response whatever appeared on prednisone treatment, as has been noted in the case of chronic thrombocytopenia reported earlier (22). On the other hand a similar good effect of splenectomy was observable.

The cause of thrombocytopenia in infectious mononucleosis, and the role of the spleen, have been widely discussed. A suggestion has been made that hypersplenism is the main cause (8), along with the presence of an increased number of immature megakaryocytes in bone marrow as evidence of abnormal maturation. Nevertheless, the marrow finding is also explicable as a hyperactivity compensating for rapid disappearance of the platelets. The results obtained in the investigation with ^{51}Cr-labelled platelets indicate that the last mentioned mechanism is more likely. The rapid rise in platelets after splenectomy may be attributable to removal of the splenic trap and the distribution of a normal total number of platelets within a smaller volume. Normally about one third of the platelets are concentrated in the spleen, in equilibrium with the remaining platelet mass in the vascular system (3). In surgically removed spleens it has been found that the splenic platelet pool is disproportionately greater than the blood it contains (18). The alteration in the state of equilibrium in infectious mononucleosis and other viral diseases seems to be dependent upon immunological features. By the application of a platelet aggregation test (19) it has been shown that soluble viral antigen-antibody complexes may damage the platelets and induce thrombocytopenia. This has been demonstrated for inter alia, herpes, rubella, measles, cytomegalo and group B arborvirus antigens in the presence of circulating antibodies (15 19 20). This mechanism explains the short-duration thrombocytopenia normally observable during the viraemic phase of viral diseases. However it does not explain why in some cases thrombocytopenia persists for a lengthy period. In these cases the underlying mechanism is just as obscure as that of chronic idiopathic thrombocytopenic purpura. In some cases direct positive platelet agglutination tests have been demonstrated (9 14) and a positive indirect platelet antiglobulin consumption test (23) as well as a positive direct test (5) have also been recorded. In one case an initially high IgA concentration was found, which was normalized within 4 weeks (14). In the present case no alterations occurred in the immunoglobulin concentrations. Obscurity still prevails in regard to the true significance of the platelet agglutinins and the platelet antiglobulin consumption tests.

As a rule the diagnosis of infectious mononucleosis is not difficult. However when thrombocytopenia is the first sign of the disease, the occurrence of atypical lymphocytes in the peripheral blood may suggest acute leukaemia. Examination of bone marrow specimens and determination of the heterophil agglutinin titre exclude leukaemia. If the initial symptoms of infectious mononucleosis are overlooked, and the thrombocytopenia persists, chronic idiopathic thrombocytopenic purpura may be erroneously diagnosed. Consequently importance is attached to exclusion of the possibility of infectious mononucleosis in such cases.

Although thrombocytopenia may on occasion develop in infectious mononucleosis, the prognosis is almost always good. If bleeding is severe, corticosteroids seem to be the drug of choice: most

cases have promptly responded with increased platelet counts. However fatal cases are reported, and it is thus justifiable to perform splenectomy if dangerous bleeding persists or if the platelet count remains at a dangerous level. In all the cases reported, rapid and lasting normalization of the platelet level has been achieved.

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THE RENAL EXCRETION OF ACETOACETATE AND 3-HYDROXYBUTYRATE DURING ABSOLUTE FASTING

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Abstract. During absolute fasting with increasing ketosis the renal excretion of acetoacetate (AA) and 3-hydroxybutyrate (3-HB) has been in principle found to be identical. At low plasma concentrations the urinary excretion rate was near 0. With AA concentrations greater than approximately 0.09 mmol/l and 3-HB concentrations greater than approximately 1 mmol/l the urinary excretion rate of both AA and 3-HB increased rectilinearly with the plasma concentration, indicating that clearance was constant. AA/creatinine clearance ratio and 3-HB/creatinine clearance ratio increased, accordingly from approximately 0 to an average of 0.15 and 0.19 respectively. These results are in agreement with the view that at low plasma concentrations the ketone bodies are reabsorbed almost completely whereas an amount corresponding to about 1/5 of the filtered amount is excreted at higher plasma concentrations. Binding of ketone bodies to plasma proteins was not found in filtration studies and therefore both AA and 3-HB are apparently completely ultrafiltrable.

In spite of the fact that determination of the excretion of ketone bodies in the urine is used extensively in clinical medicine, the knowledge of the renal excretion of acetoacetate (AA) and 3-hydroxybutyrate (3-HB) is scanty. Neither have studies been performed of the possible binding of ketone bodies to plasma proteins, which form the basis for any investigation of the clearance of these substances.

In order to elucidate these questions, we have determined the urinary excretion rate of AA and 3-HB at various concentrations of these substances in plasma during absolute fasting and have studied whether ketone bodies can be considered as fully ultrafiltrable.

MATERIAL AND METHODS

Three overweight patients, two women and one man, aged 20–35, were treated with absolute fasting for 10–12 days. All patients had normal urinary sediment on the basis of urinary microscopy and normal 24-hour clearance of creatinine. No patient had proteinuria. Regularly during the course of fasting, the patients were studied in the morning before arising. During successive periods of about 30 min each, urine was collected without catheter. At the beginning and the end of each period venous blood sample was collected in tube containing EDTA as anticoagulant. The tubes were immediately placed in an icewater bath and centrifuged in an icefilled container.

The concentration of AA and 3-HB was then determined in both plasma and urine using an enzymatic micromethod (8). The concentration of creatinine was also determined in both plasma and urine using the photometric picrinic acid method after adsorption on Lloyd's reagent.

One hour before the studies the patients drank 500 ml water. Thereafter they drank 200 ml water at the beginning of each period after completion of voiding and venous puncture. Only periods with diuresis of 1–12 ml/min (the physiological range of diuresis (6)) were considered adequate for study. On this basis there were 38 study periods in all. The length of the study periods was relatively short in order to ensure that the spontaneous decarboxylation of AA in the urine was minimal and to keep the concentration of ketone bodies in the blood at an almost constant level throughout the whole period. Mean ketone body concentration in plasma was calculated according to the following formula:

$$\frac{P_1 - P_2}{2.3 \log \frac{P_1}{P_2}}$$

where P_1 and P_2 are the ketone body concentrations at the beginning and the end of each study period.

In order to study the possible binding of ketone bodies to plasma proteins, venous blood was collected under mineral oil in tube with EDTA as anticoagulant. After centrifugation plasma was pipetted off under mineral oil into two tubes. One tube was placed at room temperature; ultrafiltration of plasma in the other tube was undertaken at room temperature and under atm. pressure through Pellicon PSAC 025 collodium membrane, nominal molecular weight cut-off 1 000 (Millipore Corp., USA).

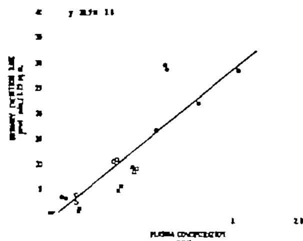


Fig. 1. Correlation between urinary excretion rate and mean plasma concentration of AA. O = patient 1, ● = patient 2, □ = patient 3.

The concentration of AA and 3-HB was determined in both the plasma and the ultrafiltrate. In addition the protein concentration was determined in the ultrafiltrate by means of biuret method (2).

RESULTS

For correlation and regression analysis Spearman's rank correlation and the method of least squares were used.

Excretion rate of AA and 3-HB in urine in relationship to the plasma concentration

Fig. 1 shows the excretion rate of AA in urine in relationship to the mean plasma concentration. With plasma concentrations less than 0.09 mmol/l the urinary excretion rate was 0 or very low. At higher concentrations the urinary excretion rate rose rectilinearly with increasing plasma concentrations. The correlation between the urinary excretion rate and plasma concentration was significant ($r = 0.851$, $\alpha < 0.001$). The regression line is seen in the Figure.

Fig. 2 shows the excretion rate of 3-HB in urine in relationship to the mean plasma concentration. At plasma concentrations less than approximately 0.9 mmol/l the urinary excretion rate was very low. At plasma concentrations greater than 1 mmol/l the urinary excretion rate rose rectilinearly with increasing plasma concentrations and there was a significant correlation between the urinary excretion rate and the plasma concentration ($r = 0.755$, $2\alpha < 0.001$). The regression line is given in the Figure.

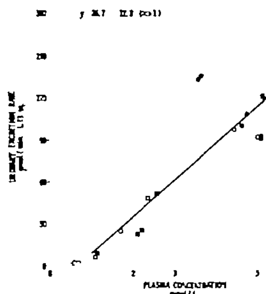


Fig. 2. Correlation between urinary excretion rate and mean plasma concentration of 3-HB. Symbols as in Fig. 1.

Relationship between corresponding plasma concentrations of AA and 3-HB

Fig. 3 shows the correlation between the concentration of AA and 3-HB in plasma specimens taken at the same point of time. The correlation was significant ($r = 0.910$, $2\alpha < 0.001$). The regression line is given in the Figure. At AA concentrations less than 0.07 mmol/l AA was dominant. On increasing concentrations the relationship between the plasma concentrations of 3-HB and

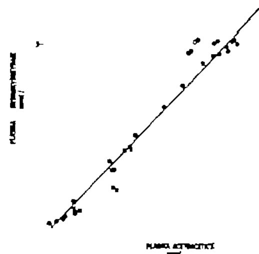


Fig. 3. Correlation between corresponding concentrations in plasma of 3-HB and AA. Symbols as in Fig. 1.

AA rose to a constant value of approximately 3.5.

Relationship between corresponding urinary concentrations of AA and 3-HB

Fig. 4 shows the relationship between concentrations of AA and 3-HB in simultaneous urinary specimens. The correlation was significant (r = 0.983, $2\alpha < 0.001$). The regression line is given in the Figure. At AA concentrations less than approximately 0.7 mmol/l AA was the dominant ketone body in the urine. At greater concentrations the relationship between the urinary concentrations of 3-HB and AA rose to approximately 4.

Ketone body/creatinine clearance ratio (Table I)

At plasma AA concentrations less than 0.09 mmol/l the AA/creatinine clearance ratio was 0–0.01. At higher concentrations in the plasma the ratio increased to values between 0.1 and 0.3 (mean 0.15). At plasma 3-HB concentrations less than 0.9 mmol/l the 3-HB/creatinine clearance ratio was 0–0.03. At greater plasma concentrations the ratio varied between 0.1 and 0.4 (mean 0.19).

Creatinine clearance

The creatinine clearance for each study period is shown in Table I. The creatinine clearance did not change characteristically during fasting and varied between 72 and 156 ml/min/1.73 m²

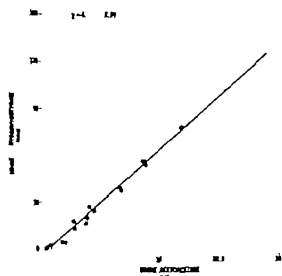


Fig. 4. Correlation between corresponding concentrations in urine of 3-HB and AA. Symbols as in Fig. 1.

Table I. *Mean ketone body concentration, ketone body/creatinine clearance ratio and creatinine clearance in each study period*

Pat. no.	Mean plasma conc. (mmol/l) AA	Mean plasma conc. (mmol/l) 3-HB	AA/creatinine clearance ratio	3-HB creatinine clearance ratio	Creatinine clearance (ml/min/1.73 m²)
1	0.044	0.100	0	0	95
	0.043	*0.077*	*0.01*	*0.01*	*151*
	0.237	0.614	0.06	0.02	155
	0.237	0.650	0.11	0.02	148
	0.543	1.69	0.13	0.10	150
	0.560	1.61	0.15	0.10	1 7
	0.879	2.74	0.13	0.17	141
	1.33	4.38	0.10	0.16	136
	1.36	4.41	0.05	0.10	147
	1.20	4.96	0.19	0.13	149
	1.18	5.17	0.11	0.14	154
	1.20	5.08	0.15	0.15	156
2	0.130	0.232	0.17	0.03	148
	0.164	0.284	0.18	0.03	102
	0.954	3.56	0.28	0.34	110
	0.956	3.60	0.28	0.36	106
	1.37	5.08	0.21	0.27	121
	1.44	*4.99*	*0.25*	*0.32*	*110*
	1.21	4.58	0.17	0.21	105
	1.24	4.68	0.24	0.23	100
	1.18	4.13	0.19	0.21	112
	1.07	3.90	0.26	0.25	84
	1.35	4.95	0.19	0.22	90
	1.42	5.09	0.22	0.28	72
	1.50	5.05	0.15	0.19	101
	1.58	5.05	0.19	0.19	84
	1.65	3.84	0.19	0.23	103
	1.63	3.97	0.21	0.16	86
3	0.061	0.112	0	0	128
	0.272	0.492	0.02	0.01	146
	0.273	0.458	0.02	0	123
	0.451	0.840	0.05	0.01	92
	0.608	1.15	0.07	0.06	132
	0.575	1.10	0.08	0.06	100
	0.689	2.10	0.11	0.09	126
	0.671	2.18	0.17	0.09	134
	0.719	2.35	0.11	0.17	118
	0.698	2.55	0.15	0.20	102

Binding of ketone bodies to plasma proteins

Table II shows the concentrations of AA and 3-HB in plasma and ultrafiltrate from four blood specimens. The concentration of both ketone bodies was somewhat higher in the ultrafiltrate than in the plasma. Thus binding of ketone bodies to plasma proteins did not take place. The protein concentration in the ultrafiltrate was 0.1–0.3 g/l, corresponding to a protein leak of 0.1–0.4%.

Table II *Concentrations of AA and 3-HB in plasma and ultrafiltrate from four blood specimens*

	Plasma (mmol/l)	Ultrafiltrate (mmol/l)	Protein conc. in ultrafiltrate (g/l)
AA	0.919	1.074	0 2
3-HB	4.98	5.39	0.2
AA	0.419	0.446	0.3
3-HB	1 71	1.84	0.3
AA	0.414	0.510	0.1
3-HB	4.50	4.55	0.1
AA	0.159	0 209	0.3
3-HB	0.560	0.592	0.3

DISCUSSION

The present study has shown that AA and 3-HB are in principle dealt with identically by the kidneys. At low plasma concentrations the urinary excretion rate was either 0 or very small. Thus the clearance was about 0. At higher concentrations the urinary excretion rate rose rectilinearly with the plasma concentration so that clearance was constant. Clearance of the ketone bodies was markedly less than creatinine clearance. AA/creatinine clearance ratio was thus less than 0 30 3-HB/creatinine clearance ratio less than 0.40

These results are in accordance with the view the ketone bodies are reabsorbed in the les during absolute fasting. The reabsorption was almost complete at AA plasma concentrations less than approximately 0 09 mmol/l and 3-HB plasma concentrations less than approximately 0.9 mmol/l. At higher plasma concentrations the excretion rate rose to a level corresponding to approximately 1/5 of the filtered amount.

Studies of the renal excretion of ketone bodies with other forms of ketosis likewise suggest a tubular mechanism of absorption. This was the case in studies in dogs on infusion of AA or 3-HB (5 7) and in the studies by Galvin et al. (1) in individuals with starvation-exercise ketosis, post-absorptive exercise ketosis and ketosis in association with a high fat diet. Thus, as in our studies, Galvin et al. found a linear relationship between the urinary excretion rate and the plasma concentration of AA whereas the urinary excretion rate of 3-HB in relationship to the plasma concentration was, in contrast to our studies, found to be curvilinear. This discrepancy could be related to different experimental conditions during the various forms of ketosis studied by Galvin et al. thus making it difficult to compare the results. It may be however that the renal excretion of 3-HB differs during the ketosis of absolute fasting from that during other forms of ketosis.

It is interesting that Rooth and Carlström (4) found in fasting patients that some individuals did not excrete increased amounts of AA in the urine in spite of a rising blood concentration. In a single patient the excretion of AA in the urine *was almost 0 in spite of a blood concentration of* approximately 1.5 mmol/l. The explanation could possibly be that efforts to prevent the spontaneous decarboxylation of AA by cooling off the urine do not appear to have been made.

It is possible that the renal handling of AA and 3-HB during absolute fasting is more complex than a simple filtration followed by reabsorption. Owen et al. (3) demonstrated by metabolic balance studies in fasting individuals that a renal uptake of 3-HB took place simultaneously with a renal production of AA and suggested that the renal handling of ketone bodies during absolute fasting is complicated by metabolism and interconversion.

The present study has also shown that the ketone bodies on filtration studies are not bound to plasma proteins. AA as well as 3-HB are therefore apparently fully ultrafiltrable. The somewhat greater concentration in the ultrafiltrate in relationship to the plasma concentration is presumably due to the Donnan effect.

ACKNOWLEDGEMENTS

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Announcement

The International Prize for Modern Nutrition of Sfr 15 000.— will be awarded in Sept. 1973 by the Central Union of Swiss Milk Producers, Berne to a scientist from one of the following countries, members of the International Dairy Federation. Argentina, Australia, Austria, Belgium, Brazil, Bulgaria, Canada, Czechoslovakia, Denmark, Finland, France India, Ireland, Israel Italy Japan Kenya, Luxemburg, the Netherlands, New Zealand, Norway Poland South Africa, Spain, Sweden, Switzerland, United Kingdom, USSR, West-Germany

The subject for the prize is *Relation between food carbohydrates and atherosclerosis (diabetes excluded)*. All scientists (chemists, physicians, biologists) who have worked in this field are eligible.

Applications. Professor M. Demole Unité de Diététique Hôpital Cantonal, CH 1211 Genève 4 Suisse, until Jan. 31 1973 with 3 copies of a) curriculum vitae, b) list of works, c) reprints of 2 or 3 papers on the theme of the prize, published in the last 5 years (no typewritten papers). These documents should be written in English, French or German or should be accompanied by a translation into one of these 3 languages. (They will not be returned to the authors.)

Information. Secrétariat du Service de Diététique Hôpital Cantonal, CH 1211 Genève 4 Suisse.



At the end of this year I shall be retiring from the editorship of this journal. Admittedly during the coming year quite a number of papers will be published which have passed through my hands and for the publication of which I am in reality responsible. But this is the last issue carrying my name as Editor on the cover

When I now look back on these 15 years and more, I immediately think of a number of changes that have taken place during this period Some of them I have already touched upon in conjunction with the centenary of the journal. Among the more important are that the journal has acquired an entirely new and modern look, that we have gone over from Summaries to Abstracts, and that we regularly publish one issue per month, making two volumes per annum.

When I took up this post, Nanna Svartz was chairman of the board of the Society for the publication of Acta medica scandinavica. On her retirement in 1969 she was succeeded by Gunnar Biörck. I should like to express to them my great gratitude for the excellent relations, free from friction of any kind, that I have had both with them and with the remainder of the board.

I am equally desirous to express my gratitude to the Redactores of the journal in the various countries for the great interest and the unstinted labour they ha e devoted to the examination and revision of the manuscripts entrusted to them. Without their invaluable help I could not have fulfilled my editorial task. No less do I appreciate the initiative they have taken for improvement of the journal. At the last meeting of Redactores at Gothenburg this summer certain improvements were suggested which it has not yet been possible to put into effect and which, therefore will be the concern of my successor

My warm thanks are due also to our subeditor Mrs Marita Afsar who carries out her exacting work with skill and grace, and to her predecessor Mrs Elisabet Whstrand, who has kindly deputized for her when required, during recent years.

The journal now has an abundant influx of manuscripts, a reflection of the seething scientific activity which prevails in the Scandinavian countries. It has been a great source of satisfaction to be able to help so many authors to have the results of their labours put into print and disseminated to the scientific world. On the other hand the large number of manuscripts has meant that space could not always be found for papers recommended by the Redactores if the time of publication was not to be unreasonably delayed. It has also meant that space has been available for only an e tremely small number of papers from countries outside the sphere of the Editorial Board.

The coverage of the journal increases year by year the number of subscribers has doubled in these 15 years, so greatly extending the circle of readers.

During my editorship, accordingly, I have enjoyed pleasant relations in all directions, I am unreservedly in favour of the improvements that have been suggested, and I am still in excellent health and in possession of undiminished powers. One may then wonder why I have decided to end the stimulating work involved in the editorship of a journal of the character of Acta medica scandinavica.

I may reply that after these 15 years I consider I have done my bit for the journal and for Scandinavian collaboration in this field. Nor in my opinion is it an advantage that an editor should remain too long at his post. He should himself realize when the time has come to hand over to younger talents who may be considered to be better able to carry on the work. Perhaps, too, I shall then have time for other matters upon which my fancy is set.

With these words I resign from the editorship with gratitude for the growing interest shown for our journal.

Birger Strandell

TO THE READERS OF ACTA MEDICA SCANDINAVICA

In 1969 the Acta medica scandinavica celebrated its centenary. The Editor, Birger Strandell, gave a survey of the activities during these first 100 years in our volume 185 (pp. 1–8) of that year. Among other things, he noted that he was the fourth in the line of Editors during these hundred years. However, with the present volume, number he is to retire after 15 years of service devoted this journal.

Behind the Acta medica scandinavica—as you may read in Strandell's paper—there is a "Society for the publication of Acta medica scandinavica. It is in my capacity as chairman of the board of this society that I am announcing Birger Strandell's retirement from his post. As from January 1973 (volume 193), Professor Jan Waldenström will succeed him as Editor with Assistant Professor Harry Boström and Professor Lars Erik Böttiger as Assistant Editors. The new Editors will introduce themselves and their plans for the future of the journal in the first issue of 1973. On behalf of the Board of the Society and of the readers of Acta medica scandinavica I wish them good luck in their new endeavour.

Birger Strandell was elected Editor in the autumn of 1957 and has thus completed more than 15 years in that post. In addition, he has served as vice-chairman of the Board of the Society. His predecessor, Professor Israel Holmgren, took farewell of his readers after 41 years of service through a public announcement that he was retiring "because of cancer prostatae. Fortunately Birger Strandell retires in good health to enjoy his *otium cum dignitate* in order to pursue new tracks with his indefatigable intellectual curiosity and fine intuition as a scientific collector.

In this issue of the Acta medica scandinavica, the last to carry his name on its front page, it behoves me, in the name of the Society, of many hundreds of authors and of many thousands of readers, to thank Birger Strandell for a very efficient, very pleasant and very smooth leadership. During his time the journal has doubled its circulation. The number of chairs of medicine in the Scandinavian countries has increased considerably and, in consequence, there has been a corresponding widening of the editorial board, the Redactores of Acta medica scandinavica. The skill and, at times, benevolent diplomacy with which our Editor has handled this situation have created a feeling of admiration and won him a great many friends. This was clearly manifested at the last meeting with the Redactores in Gothenburg in June 1972. It is indeed no mean testimony to the ability and devotion of Birger Strandell as Editor of our journal that, on his retirement, it takes three men to fill his post.

Gunnar Biörck

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HUMAN PHARMACOKINETICS OF A SULFAMETHOXAZOLE TRIMETHOPRIM COMBINATION

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Abstract. The pharmacokinetic characteristics of the fixed chemotherapeutic combination of sulfamethoxazole (SMZ) and trimethoprim (TMP) have been elucidated in 14 patients with different renal functions. The peak serum concentrations (c_{max}) after two tablets totalling 160 mg TMP and 800 mg SMZ in patients with creatinine clearances (C_{cr}) above 60 ml/min were around 40 µg/ml for potentially antibacterial SMZ, the non-active SMZ metabolites in addition contributed another 10 µg/ml. The TMP peak values averaged 1.4 µg/ml in serum. In patients with renal insufficiency the c_{max} was lower. Serum half-life ($t_{½}$) of active SMZ (SMZ_a) was 7 hours normally and only slightly changed with impaired kidney function. The $t_{½}$ for total SMZ (SMZ_t) and TMP, however, normally 12 and 13 hours respectively were increased in patients with reduced C_{cr} values. This increase was most marked for SMZ_t. The $t_{½}$ of SMZ_t began to increase around C_{cr} 30 ml/min, $t_{½}$ of TMP only at around 20 ml/min. In addition to similarities between SMZ and TMP excretion rates in normal persons, invasion phase kinetics of both compounds were of the same order. The distribution coefficient, however, was close to 2.0 for TMP but only 0.2 for total and 0.3 for active SMZ. In patients with C_{cr} above 60 ml/min, 4–5.5 hour urine samples exhibited mean SMZ_a concentration of 29.5 µg/ml and TMP concentration of 78.4 µg/ml. There were approximately 15 times more non-active SMZ metabolites than SMZ_a. All components appeared in lesser urine concentrations in renal insufficiency.

The antibacterial combination sulfamethoxazole (SMZ) and trimethoprim (TMP) has become available for antibacterial therapy. Many reports of the therapeutic response to this drug have appeared (3, 4, 6), the serum concentration profile of only a small number of subjects with normal renal function has yet been published (8, 14, 15). The consequence of impaired renal function needs careful assessment to implement the correct administration in this group of patients. It is the purpose of this communication to present the pharmacokinetics of the SMZ TMP combination with normal and with reduced renal function.

MATERIAL AND METHODS

Subjects

Fourteen patients with normal and varying degrees of depressed endogenous renal creatinine clearance (C_{cr}) were studied. The clearance values given are the mean of two determinations. The vital characteristics of the patients are recorded in Table I. The subjects received SMZ/TMP for a urinary tract infection caused by a microbe sensitive to the combination as assessed by the triple disk technique elaborated by Böhm (3).

Dosage

In the fasting state each patient was given two tablets totalling 800 mg SMZ and 160 mg TMP to be swallowed with a glass of water. Food was only allowed after 1 hour. Where possible, all other medication was withdrawn. In the cases included in this report, concomitant medication did not interfere with the assay procedure. Venous blood was sampled at 1, 2, 4, 5.5, 7 and 12 hours. Five subjects with reduced renal function also had specimens taken at 24 and 48 hours.

The serum was stored at −70°C until assayed and kept in the frozen state during transportation.

All samples were analyzed by Hoffmann–La Roche, Basel, Switzerland. Chemical assay procedures were employed, spectrophotometric for SMZ (11) and fluorospectrophotometric for TMP (13, 15).

The SMZ was determined as two fractions, the total SMZ (SMZ_t) and the portion thereof which is not acetylated, glucuronidated or transformed to sulfonic acid and consequently assumed to be potentially antibacterially active (including the protein bound portion) (SMZ_a). The assay procedure for TMP encompasses only a small portion of metabolized non-active TMP (13).

Calculation of pharmacodynamic characteristics and statistics

The calculation of the pharmacodynamic characteristics generally followed procedures detailed by Dost, ac

Table I. *Renal function and other characteristics of 14 patients*

Pat. no.	Sex	Age (y.)	Weight (kg)	Endogenous C_{cr} (ml/min)	Comment
1	♀	44	79	99	
2	♀	45	61	84	
3	♀	42	91	82	IgM component in serum of 1.2 g/100 ml
4	♀	60	56	67	
5	♂	51	92	65	
6	♂	66	97	64	
7	♀	60	76	61	
8	♀	52	61	48	
9	♀	67	46	32	
10	♂	37	64	22	
11	♀	48	76	7	
12	♀	47	63	7	
13	♂	46	80	3	
14	♂	38	78	3	
Normal range				70	

cording to first order open compartment model (7). The regression of the excretion curves past the peak serum values (max) was achieved by the least squares regression procedure (16). Serum half-life ($t½$) was related to the regression coefficient (*b*):

$$t½ = \frac{\log 2}{-b}$$

The excretion coefficient (k_2) as equal to ln 2/$t½$.

The fictive serum concentration (c_0) that would have resulted at the time zero if the same dose as given ally had been injected and an even distribution reached immediately as achieved by the principle of corresponding areas (*F*) under serum concentration curves resulting from corresponding doses

$c = Fk$

where *F* is the sum of *F* (the area resulting during the observation period) and *F* (the area calculated for the remainder of the period until zero serum concentration using the formula *F* k_2, here c_n is the serum concentration at the final blood sampling as calculated from the least squares regression for the excretion curve). The invasion constant (k_1) as calculated as $k_1 = k_2/(1-c_0)$, here is the intercept of the excretion phase regression and the ordinate

The distribution coefficient as obtained as described by Dost (7). Estimation of the lowest serum concentration occurring after repeated dosage, min, at the end of dosage interval τ was done by simpler mathematical transformation (1) of the simplification developed by Dettli (12). Calculation of the maintenance dose (*D'*) necessary for the achievement of serum concentrations similar to those of the initial dose was done according to Bergan (2) on the basis of the

$$D' = \frac{DT}{T-1} \quad \text{where} \quad = \frac{\tau}{t½}$$

This simplification is valid hen relatively low terminal serum concentrations are found.

C_{cr} has been corrected to body surface area 1.73 m².

Differences in sample means were assessed by the Student' *t*-test,

$$t = \frac{\bar{x}_1 - \bar{x}_2}{} \sqrt{\frac{}{n}}$$

where $\bar{x}$ and $\bar{x}_2$ are sample means, is standard deviation and the number of observations.

RESULTS

Serum half-life ($t½$)

From Fig. 1 it is discernible that for SMZ_t there is a precipitous increase in $t½$ as C_{cr} becomes successively reduced below approximately 30 ml/min. The nearly indefinite $t½$ values measured for C_{cr} ≤ 10 ml/min suggest that the SMZ metabolites are excreted almost exclusively through the urine. The inactivation of SMZ_a must, however proceed by non-renal mechanisms. The $t½$ for SMZ_a is practically uninfluenced by alterations in kidney function, although renal insufficiency entails a larger variation (Fig. 2). The $t½$ for TMP as well is dependent on renal function (Fig. 3), although this is less marked than for SMZ_t the rise in $t½$ with increasing renal insufficiency begins at a lower C_{cr} value.

From the above it will appear that for studying $t½$ patients with a renal function above C_{cr} = 60 ml/min may with an ample margin of safety be allocated to the normal material

The mean $t½$ values for the three entities SMZ_a, SMZ_t and TMP are listed in Table II

In the group of four patients with C_{cr} between 3 and 7 ml/min, the $t½$ for SMZ_t varied between 79.2 and 273.6 hours (Fig. 1) The $t½$ for TMP in the same group was between 17.5 and 215.0 hours (Fig. 3).

On the basis of the $t½$ values for patients with $C_{cr} > 60$ ml/min the calculated mean k_2 value for SMZ_t was 0.1066 and for TMP 0.0661

It was found that patient 3 exhibited a $t½$ for TMP which, being 29.3 hours, was more than twice the mean estimate for $C_{cr} > 60$ ml/min. The $t½$ for SMZ_a and SMZ_t were 4.8 and 5.4, respectively

Peak serum concentrations (c_{max})

The c_{max} for the patients with $C_{cr} > 60$ ml/min and $C_{cr} < 10$ ml/min are indicated in Table III. It is seen that the values for TMP were within

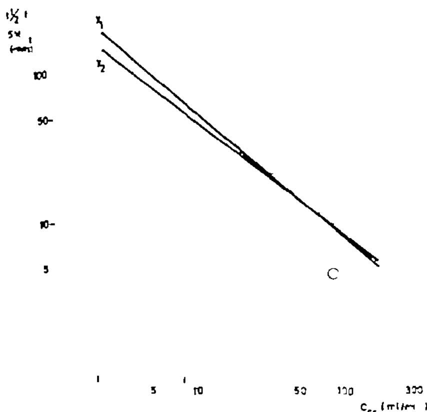


Fig. 1. Relationship between C_{cr} and $t_{1/2}$ of total SMZ, in 14 patients. Regression lines have been calculated for our study (y_1) and for these observations together with those of another study (12) (y_2). ○ = the myeloma patient (no. 3).

$y = 2.5697 - 0.8271x$, $r = 0.9017$, $n = 14$
$y = 2.4167 - 0.7392x$, $r = 0.8560$, $n = 34$

Patients with $C_{cr} < 1$ and with infinite $t_{1/2}$ values were excluded. The x and y are the $^{10}\log$ to the C_{cr} and $t_{1/2}$, respectively.

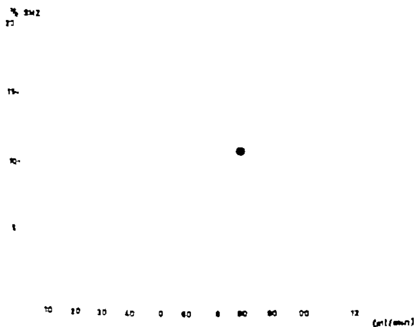


Fig. Relationship between C_{cr} and $t_{1/2}$ of SMZ, in 14 patients. ● = the myeloma patient (no. 3).

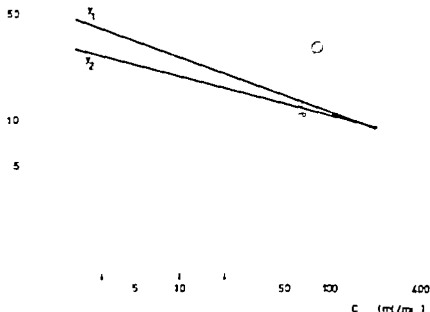


Fig. 3. Relationship between C_{cr} and $t\frac{1}{2}$ of TMP. Explanation of Figure otherwise as for Fig. 1.
$y = 1.7740 - 0.3631x$, $= 0.5489$ $n = 14$
$y = 1.5425 - 0.2576x$, $= 0.5427$ $n = 38$

Patients with $C_{cr} < 1$ and with $t\frac{1}{2} = \infty$ were excluded. The x and y are the "log to the C_{cr} and $t\frac{1}{2}$, respectively. ⊕ = the myeloma patient (no. 3). Explanation of y and y_2 given in Fig. 1.

the same range whereas for SMZ there were lower values in patients with a reduced renal function. This was most pronounced for SMZ_a, which was reduced in the group with $C_{cr} < 10$ ml/min.

Due to the relatively few samples obtained during the invasion phase it was difficult to make an exact estimate of the time of occurrence for the maximal serum concentration (t_{max}) but for SMZ_t in this study it occurred mostly at 4 hours, occasionally at 2 hours. In two patients with $C_{cr} < 10$ ml/min the t_{max} for SMZ_t did not occur until 5.5 and 7 hours. The t_{max} for TMP was 2 hours in all but one patient.

Table II. *Mean $t\frac{1}{2}$ of SMZ_a, SMZ and TMP in seven patients with renal function above 60 ml/min*

	Mean $t\frac{1}{2}$	Range	S.D.
SMZ_a	7.0	4.1–9.0	3.4
SMZ_t	11.8	5.4–22.3	5.5
TMP	13.4	7.0–29.8	7.6

It should be noted that the serum concentration of SMZ_a, SMZ_t and TMP in patient 3 were close to the mean for the group with a $C_{cr} < 10$ ml/min.

Serum concentrations of SMZ and TMP

The mean serum concentrations of SMZ_t and TMP in the patients with $C_{cr} > 60$ ml/min is discernible from Figs. 4 and 5. Particularly dur

Table III. *Variation in c_{max} of SMZ_a, SMZ_t and TMP in seven patients with C_{cr} above 60 ml/min and in four patients below 10 ml/min*

	Mean max	
	C_{cr} 60 ml/min	C_{cr} < 10 ml/min
SMZ_a	39.7 (34.0–49.0)	30.3 (19.6–42.4)
SMZ_t	50.7 (48.1–57.7)	44.2 (34.2–59.4)
TMP	1.36 (1.13–1.60)	1.33 (0.86–…11)

Range given within parentheses.

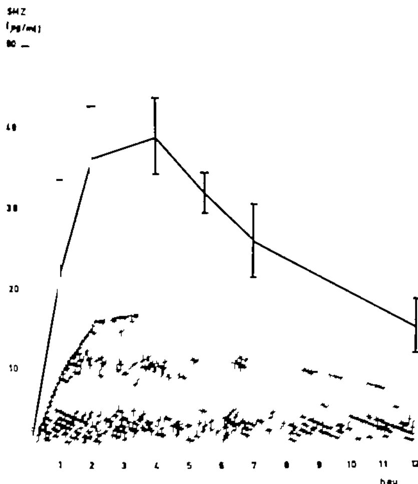


Fig 4 Mean serum concentration f SMZ_s for 7 patients ith C 60 ml min. The vertical bars indicate S D The shaded area represents the amount of non-protein bound SMZ_s.

ing the invasion phase and first period after equilibrium there was considerable variation in the serum levels achieved within the group.

It is to be added that the serum concentrations of SMZ_u, SMZ_f and TMP in patient 3 were close to the mean for the group with $C_{cr} < 10$ ml/min.

Invasion coefficient (k_1)

On the basis of the law of corresponding areas, estimates have been made for the invasion constants of SMZ_u SMZ_f and TMP In the subjects with $C_{cr} > 60$ ml/min the mean k_1 for TMP was 1.7001 for SMZ_s 1 1117 and for SMZ_f 1.0168. The largest variation was found for TMP for which the extreme k values were 0.4200 and 4 8207

The k_1 for the SMZ_s and SMZ_f varied between 0 4300 and 2.4331

Distribution coefficient (Δ)

The distribution coefficient appeared to be uninfluenced by renal function (Table IV) It is notable that, whereas the mean Δ was 0 1981 for SMZ_f and 0.2770 for SMZ_u the mean for TMP was considerably higher than unity 1 7464

Concentrations in urine

The concentrations of SMZ_u SMZ_f and TMP attained in the urine are recorded in T ble V It is seen that this diminished with decreased renal function. Patient 3 who had the lowest urine concentration, also had a high uresis, 240 ml, during the 90-min examination period.

In the patients with $C_{cr} > 60$ ml/mn the mean urine concentration was 29.5 µg/ml for SMZ_s and 78.4 µg/ml for TMP If patient 3 is ex cluded, his concentrations being markedly dif

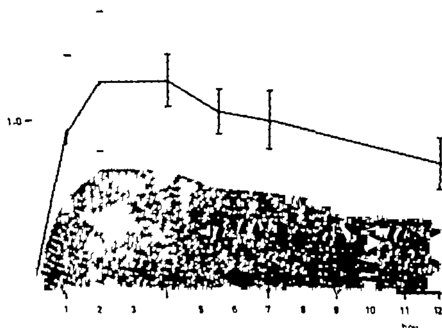


Fig 5 Mean serum concentrations of TMP in 7 patients with $C_{cr} > 60$ ml/min. The vertical bars indicate S.D The shaded rea re presents the amount of nonprotein bound TMP

ferent from the rest, the values become 36.4 and 1.5 µg/ml, respectively

The relative amounts of SMZ_a to SMZ_f with ormal and reduced renal function are also important. As a reflection of the increased ½ of SMZ_f in renal insufficiency the subjects with $C_{cr} > 60$ ml min had a mean SMZ_f SMZ_a ratio of 16.2, whereas in the group below 10 ml/min it was 3.29

Fraction of TMP relative to SMZ

The in vitro examinations mostly on *Enterobacteriaceae* and staphylococci, have shown that, on the average the ratio between the smallest quantities of TMP and SMZ_a which synergistically inhibit sensitive bacteria is 1 20 to 1 25 The actual concentration ratios encountered in each of the serum and urine samples examined have been calculated.

In the serum of patients with $C_{cr} > 60$ ml/min the mean TMP/SMZ_a ratio reached a maximum of 1 38 at 2 hours and subsequently fell gradually to 1 21.3 at 12 hours. In the four patients with $C_{cr} < 10$ ml/min the ratio fell gradually from 1 22.2 at 2 hours to 1 9 7 at 24 hours and 1 6.0 at 48 hours. These ratios pertain to the sum of the protein bound and unbound portions of SMZ_a and TMP The relationships between t_{max} for the SMZ_a and TMP in the patients with $C_{cr} > 60$ ml/min have fluctuated between 0.08 ($C_{cr} = 81$ ml/min) and 6.91 ($C_{cr} = 67$ ml/min) In the group of four patients with C_{cr} less than 10 ml/min, however the ratios were 0.04, 0.08, 0 45 and 0 62, i.e. on the whole considerably lower

In the urine samples taken between 4 and 7 hours after tablet intake (divided into two 1 5-hour samples for most individuals), the ratio TMP/SMZ_a in the seven patients with $C_{cr} > 60$ ml/min was 1 25 on an average however if patient 3 with a ratio of 1 12 is excluded the ratio becomes 1 0 85 this is a more meaningful figure since the values in the six remaining patients ranged from 0 3 to 3 4

It is seen that also in individuals with a renal function below 10 ml/min C_{cr} the serum concentration of SMZ_a exceeds the TMP

Table IV *Distribution coefficient of SMZ_u, SMZ_t and TMP in 14 patients with varying renal function*

Pat no.	Endogenous C_{cr} (ml/min)	Distribution coefficient TMP	SMZ_u	SMZ_t
1	99	1.3105	0.1650	0.1674
2	84	1.6559	0.3015	0.2288
3	82	1.5427	0.1589	0.1187
4	67	1.7128	0.3293	0.2374
5	65	1.2758	0.2081	0.1488
6	64	3.3995	0.1752	0.1445
7	61	1.2966	0.1857	0.1717
8	48	1.9069	0.2261	0.1483
9	32	1.8131	0.3337	0.2340
10	22	1.9904	0.3052	0.2273
11	7	1.3634	0.2940	0.2401
12	7	1.1952	0.2556	0.2125
13	3	2.3474	0.4982	0.2411
14	3	1.6401	0.4426	0.2533
Mean		1.7464	0.2770	0.1981
SD		0.5757	0.1019	0.0447

Indications of accumulation

In order to assess the possibility of accumulation of the TMP and SMZ compounds, the least serum concentrations obtained after an infinite number of doses ($c_{min,\tau}$) given at fixed intervals (τ) were calculated. Table VI shows that, whereas the mean for TMP $c_{min\ 12}$ is 1.61 and $c_{min\ 24}$ is 0.57 for patients with $C_{cr} > 60$ ml/min, there is a marked increase in c_{min} on reduction of C_{cr} below 10 ml/min. The mean values for SMZ_t were $c_{min\ 12} = 59.6$ μg/ml and c_{min} = 19.4 μg/ml. The patients with renal insufficiency exhibited markedly increased values.

Table V *Urine concentration during the period between 4 and 5.5 hours after drug intake*

Pat. no.	C_{cr}	Urine concentration (μg/ml) TMP	SMZ_u	SMZ_t
1	99	88.1	—[a]	—
2	84	172.2	69.2	1 016.4
3	82	0.18	2.2	14.1
4	67	102.9	15.9	583.4
5	65	57.4	22.0	374.0
6	64	120.7	38.4	497.2
7	61	27.6	—	—
8	48	74.9	122.6	430.3
9	32	49.5	48.8	422.4
10	22	15.6	5.4	109.8
11	7	2.2	25.1	47.5
12	7	1.78	21.4	40.5
13	3	4.1	9.0	38.0
14	3	7.1	11.4	57.9

[a] The sample disappeared in the analytical laboratory before SMZ_u and SMZ_t determinations.

Table VI. *Calculated serum concentration ($c_{min,\tau}$) of SMZ_u, SMZ_t and TMP after an infinite number of doses immediately before medication with dosage intervals (τ) of 12 and 24 hours*

Pat. no.	C_{cr} (ml/min)	TMP −12	TMP −24	SMZ_u −12	SMZ_u −24	SMZ_t −12	SMZ_t 24
1	99	0.68	0.16	61.4	1.1	29.4	7.3
2	84	1.87	0.66	28.6	8.1	47.1	14.7
3	82	3.53	1.53	11.7	1.7	20.3	3.6
4	67	1.45	0.46	21.6	5.4	64.7	22.1
5	65	1.43	0.48	20.6	5.1	70	17.5
6	64	1.08	0.35	27.6	7.5	49.4	15.6
7	61	1.19	0.35	29.9	7.7	135.6	55.3
8	48	2.53	1.00	36.6	10.2	80.2	25.8
9	32	1.93	0.64	47.2	15.2	168.8	69.8
10	22	1.37	0.47	40.7	13.5	130.0	55.9
11	7	3.91	1.96	45.3	16	1 480.6	705.0
12	7	3.49	1.34	29.9	8	55.3	63
13	3	3.26	1.44	25.9	9.3	376.9	177.9
14	3	2.27	0.89	23.1	7.7	493.6	234.0
Mean for C_{cr} 60 ml/m		1.61	0.57	28.77	5.23	57.60	19.44
S.D of above mean		0.93	0.45	15.69	2.86	37.95	16.99

Indications of accumulation are also obtained from calculations of the maintenance dose (D') necessary to achieve serum peaks of the same size as after the initial dose (Table VII). The maintenance doses for the subjects with marked renal impairment are markedly lower than those for patients with normal function.

DISCUSSION

Fixed combinations of antibiotics should usually be avoided. In the present instance two compounds were combined since they acted synergistically on the same pathway. Thereby a widening of the antibacterial spectrum was achieved.

Due to the sequential blockade achieved by SMZ and TMP a quantitatively fixed combination of SMZ/TMP has become available. Clinically various ratios and doses of the two compounds have been investigated pointing to an optimal TMP/SMZ ratio of 1 : 5 (4, 5). When a 1 : 5 ratio has been found most suitable in patients with normal organ functions, this must have, besides a bacteriological explanation, also pharmacokinetic reasons.

Table VII. *Calculated maintenance doses (D') of SMZ SMZ$_f$ and TMP necessary to achieve peak serum values equal to that after a single dose with dosage intervals* (τ) *of 12 and 24 hours*

Pat. no.	C_{cr} (ml/min)	TMP τ=12	TMP τ=24	SMZ$_a$ τ=12	SMZ$_a$ =24	SMZ$_f$ τ=12	SMZ$_f$ τ=24
1	99	111.2	145.1	693.0	785.8	537.2	713.7
2	84	73.3	113.1	482.9	674.3	439.0	637.0
3	82	38.9	68 4	600.4	775.6	627.8	762.9
4	67	85.6	125.4	534.2	711.8	385 4	585.2
5	65	78.2	118.2	535.8	712.7	361.8	560.1
6	64	84.3	124.2	504.1	690 6	428.7	627.8
7	61	92.2	131.3	523.7	704.6	249.0	420 0
8	48	56.3	92.8	490.6	680 4	419 4	619.0
9	32	79.8	119.8	419.5	619.0	244.4	412.4
10	22	76.4	116.5	401.0	601 1	236.6	396.6
11	7	6.1	11.8	355.8	553 4	23.3	47 4
12	7	60.5	98.1	499.2	686.9	78.0	148.0
13	3	33 1	59 4	349.5	546.7	79.2	151 1
14	3	56.9	93.5	400.2	600.1	61.3	117.9
Mean for pats. 1 7		80.5	118.0	553 4	722.1	432.7	615.2
S.D of above mean		22.0	24 1	71.5	42.1	122.5	111.2

Considering the excretion dynamics of the two compounds the combination appears suitable with a normal renal function the $t½$ for both SMZ$_f$ and TMP are of the same order (12–13 hours) Although the $t½$ of SMZ$_f$ and TMP are similar when renal function is good, both $t½$ values increase with lower clearances. The increase is most pronounced for SMZ$_f$. This was obvious both from the steeper slope of the SMZ$_f$ log-log regression between C_{cr} and $t½$ and from the circumstance that the precipitous rise in $t½$ for SMZ$_f$ starts at a higher C_{cr} (ca. 30 ml/min) than for TMP (ca. 20 ml min) Consequently with low renal functions, the difference in the excretion of the two compounds becomes considerable. This is readily apparent also from Tables VI and VII which indicate drug accumulation at the lowest C_{cr} values as judged both by the calculated serum concentrations immediately before the next dose and the size of the repeat dose necessary to achieve the same c_{max} as after the first dose. Consequently to guard against toxic accumulation, it is important that dosage is changed according to renal function. The charts of Figs. 1 and 3 *may* be employed to estimate the most probable $t½$ for each drug component when the renal function of a patient has been determined, thereby allowing selection of the most appropriate dosage intervals. The regression between $t½$ and C_{cr} has been calculated for our own patients and on these data together with the findings from a Swiss-Norwegian collaborative study in which we have participated (1) Combining the results of the both studies more dependable charts for clinical use are obtained.

Two aspects are of particular importance. The *first is the precipitous increment in $t½$ seen upon* reduction of glomerular filtration below a certain point. With SMZ$_f$ $t½$ started to increase at C_{cr} values of about 30 ml/min whereas for TMP the rise started at approximately 20 ml/min. Secondly the range of variation in $t½$ for SMZ$_f$ and TMP was greater within the group of subjects with markedly reduced renal function than for subjects with normal function. This was not particularly evident from the charts of our patients alone, but became obvious when the Swiss-Norwegian study was considered this rendered a total of 51 surveyable patients. The consequence of these findings is that the prediction of $t½$ for SMZ$_f$ and TMP from available C_{cr} is less precise for patients with low C_{cr} values than for those with adequate functions. The increased variation in $t½$ may be due to the reduced accuracy for C_{cr} estimations in extreme renal insufficiency and in the larger differences in acid-base states, the function of other organs, and of comedication found within this particular group. It follows, though, that reduction in renal function necessitates changes in dosage schedules to avoid drug accumulation.

Between C_{cr} values of 15 and 30 ml/min two tablets may be given every 24 hours. Below 15 ml/min, however one should refrain from using the *fixed* combination altogether This is because the rate of excretion for SMZ$_f$ and TMP at C_{cr} values below 15 ml/min is so dissimilar that the two components cannot be given with equal time intervals. A more frequent dosage of TMP would be needed, since otherwise the TMP serum concentrations indicate that the TMP towards the end of the dosage intervals is present in such a negligible amount that the therapy for all practical purposes becomes converted to a therapy with the sulfonamide alone. The relative unpredictability of $t½$ in these very patients further limits the use of the *fixed* SMZ/TMP combination at C_{cr} values below 15 ml/min.

In contrast to the situation for SMZ$_f$ and TMP

the $t\frac{1}{2}$ for SMZ_a was largely uninfluenced by the renal function (Fig. 2). This reflects the circumstance that elimination of SMZ_a depends largely on non-renal mechanisms, since in healthy humans only 15% of the ingested SMZ is excreted during the first 96 hours (14). Following biotransformation elsewhere, the antimicrobially nonactive metabolites (acetylates and glucuronides) are readily excreted by the kidneys. Although this has not been the focus of present attention, one would expect that malfunction of other vital organs, such as the liver would change the $t\frac{1}{2}$ for SMZ_a.

It is pertinent to note that the $t\frac{1}{2}$ values found by others have been within the same range as for the normal renal function in our study. Bushby and Hitchings (6) found the $t\frac{1}{2}$ for TMP to be 13.3 hours after oral dosage in four patients, and after i.v. administration to six patients 11.6 hours. Kaplan et al. (8) noted in two subjects $t\frac{1}{2}$ values for TMP of 17.3 and 15.0 hours, no information (such as pathophysiological states) was given to explain the longer $t\frac{1}{2}$ values. Schwartz and Ziegler (15) noted in four persons $t\frac{1}{2}$ values of 6.9 9.8, 12.0 and 12.0 hours for TMP as assessed by radionucleotide techniques. Schwartz and Rieder (14), after giving the combination tablets, noted a $t\frac{1}{2}$ for TMP of 8.6 (range 4.8–11.3) in ten persons and a $t\frac{1}{2}$ for SMZ_t of 9.0 (7.7–10.6) hours in seven. Bünger et al. (5) found $t\frac{1}{2}$ for SMZ given alone to be 11.0 in ten persons and 9.1 in four others. Rieder et al. (12) indicated $t\frac{1}{2}$ to be 11.0 for SMZ.

The present study showed a greater individual variation for TMP than for SMZ. Such a trend has been noted previously (14) but, in our study *patient 3* particularly accentuates this phenomenon. In spite of a C_{cr} within the normal range the $t\frac{1}{2}$ was more than doubled compared to normal. The *explanation is most likely associated* with a concomitantly raised serum IgM component. A similar constellation was observed for another patient in the collaborative Swiss-Norwegian study (12). With repeated doses toxic accumulation of TMP would have resulted. The SMZ/TMP combination is most likely contraindicated when serum IgM is raised. The $t\frac{1}{2}$ for SMZ in this patient was approximately 1/3 of the estimated mean for normal renal functions.

Due to the importance of predicting antimicrobial effect, serum concentrations receive particular interest. In our patients with C_{cr} above 60 ml/min the mean peak serum concentration of SMZ_a was slightly less than the value obtained in four persons studied by Schwartz and Rieder (14) (39.7 vs 53.8 μg/ml). TMP concentrations in our patients were also slightly less than those found in the other study (1.36 vs 1.58 μg/ml). The peak concentrations were somewhat lower in patients with low C_{cr} values.

The TMP urine levels for patients with C_{cr} above 60 ml/min were higher than the SMZ_a concentrations. The reverse applied when C_{cr} was below 10 ml min. There was a tendency towards lower urine concentrations with reduced renal function. It is interesting that the ratio between SMZ_a and SMZ_t was distinctly lower with normal renal function than in renal insufficiency. In urine the ratio between TMP and SMZ was considerably different from 1 : 20 to 1 : 40 which has yielded optimal in vitro efficacy of the combination against sensitive strains. However in serum the concentration ratio was fairly close to that optimum. Relatively less SMZ_a was found with reduced renal function. This is the direct consequence of the reduced glomerular filtration under these circumstances and is not in contrast to the finding that $t\frac{1}{2}$ for SMZ_a was unrelated to renal function, whereas the $t\frac{1}{2}$ for TMP was increased in renal insufficiency. The myeloma patient, in spite of normal serum concentrations, also had aberrant urine findings consisting of markedly reduced urine concentrations for all compounds. The high urine volume in this patient certainly was contributory.

Within the framework of the present investigation, estimates of the k have been made. Due to the spacing of sampling during the invasion phase the calculation has fair precision. The accuracy of the k values, though, is increased *by the slowness of the event of* absorption. It is interesting to note that for SMZ Bünger et al. (5) found $k = 2.06$ in serum of ten and $k = 0.75$ in plasma of four subjects. Reber et al. (9) after i.v. and oral administration of 1 g SMZ, found a $k = 0.56$. Our values were about 1.00. The k for TMP was of the same order although the individual variation was larger. Thus it appears that the two compounds in combination match each other also in terms of invasion phase kinetics.

In A, on the other hand, there was a difference

between TMP and SMZ, in that the former was associated with higher coefficient values. The Δ value for TMP was close to 2, for SMZ_a 0.3 and for SMZ_f 0.2. The variation in calculated distribution was highest for TMP The finding that tissue concentrations of TMP generally exceeded SMZ (14) may explain the higher Δ. Renal function was of no consequence for Δ.

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HEPATIC ENCEPHALOPATHY EVALUATED BY AUTOMATIC PERIOD ANALYSIS OF THE ELECTROENCEPHALOGRAM DURING LACTULOSE TREATMENT

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Abstract. Eleven patients with liver cirrhosis and operative portacaval shunts have been followed clinically and by automatic period analysis of the EEG to study the effects of lactulose on the cerebral manifestations of liver disease. In three patients disturbances of consciousness of hepatic coma character were relieved, manifested by an increase of the dominant EEG frequency and a shift in the frequency spectrum towards normal. The improvement was evident within two to five days and was maintained for two days after withdrawal. Eight patients with varying degrees of intellectual impairment, but without disturbances of consciousness, did not improve and the EEG analysis was unchanged except in one with a subnormal dominant frequency. Thus patients with hepatic encephalopathy manifested by disturbances of consciousness and reduction in the dominant EEG frequency responded to lactulose therapy.

In 1966 Bircher et al. (2) reported that lactulose improved psychic functions by preventing coma in patients with severe chronic encephalopathy following liver cirrhosis and portal-systemic shunting of blood. Other investigators (4, 5, 6, 13, 17) have similarly observed an action ascribed to alterations in the production of cerebrotoxic substances and their distribution between large bowel and blood (1, 7). Recently the cerebral metabolic oxygen consumption rate was found to increase from subnormal to normal levels in patients with encephalopathy when treated with lactulose (6). Clinical data and EEG findings, however, were not recorded.

EEG abnormalities have been found to be well correlated with grades of neuropsychiatric disturbances preceding hepatic coma (9), and in three patients with severe encephalopathy and reduced dominant frequency of the EEG Rorsman and Sulg (11) found that clinical improvement was paralleled by an increase of the dominant EEG frequency. Such an increase may precede clinical improvement by 48 hours (5).

To define more clearly the indications for lactulose treatment we studied EEGs by automatic period analysis in 11 patients with liver cirrhosis and operative portacaval shunts under periodic treatment with lactulose.

MATERIAL AND METHODS

Eleven patients (Table I) were given lactulose (Duphalac® syrup 50% $^{w}/_{v}$, 66% $^{w}/_{w}$), 60–120 ml per os per day (Table II), as required to obtain two or three soft motions daily.

In a pilot study patient 1 was followed over a period of two months (Fig. 1). Eight patients were studied for four weeks: twice before treatment and twice during treatment at an interval of four to five days, and finally nine days after discontinuation. In two patients (nos. 10 and 11) control and treatment periods were reversed, because lactulose therapy had been instituted some time before the study began. Each patient acted as his own control.

All studies were made between 10.30 and 11.30 a.m. and outpatients were instructed not to take sedatives or sleeping tablets. Other medical prescriptions were taken as usual. The patients had breakfasted two or three hours before study and all were on normal diet.

Clinical evaluation was made with reference to daily practice of normal skills, to occupation, and to changes in general condition and sleep patterns (Table II).

Arterial blood ammonia measurement was done in connection with EEG examinations in patients 2–10 using the microdiffusion spectrophotometry method of Brown et al. (3). Analyses of the sample, blank and standard, were made in triplicate. The coefficient of variation was 3.–%, normal range 40–125 μmol/l.

Part of this work was presented at the 4th World Congress of Gastroenterology, July 1970, Copenhagen.

Table I. *Laboratory data from 11 patients with liver cirrhosis treated with lactulose (Duphalac®)*

Figures within parentheses are normal values

Pat. no.	Sex	Age (y.)	Months after portacaval operation	Serum bilirubin (mg/100 ml) (<1.3)	P & P time (>65)	Alkaline phosphatases (KA units) (<13)	Serum albumin (g/100 ml) (>3.7)	Serum γ-globulin (g/100 ml) (<1.3)
1	♂	56	68	1.3	33	22	3.7	1.8
2	♂	55	8	1.2	39	13	3.0	1.4
3	♂	59	9	1.5	41	12	3.6	1.9
4	♀	55	38	1.1	81	31	3.7	2.0
5	♂	50	29	1.5	51	11	3.3	1.8
6	♀	63	26	3.9	71	39	3.7	1.2
7	♀	66	24	1.4	52	9	4.0	1.9
8	♂	42	36	1.5	66	2	4.2	0.9
9	♀	77	11	1.7	30	11	4.2	1.1
10	♀	64	19	0.6	65	10	3.2	1.1
11	♀	65	4	3.9	64	15	3.7	1.0

Electroencephalography

EEG traces were run for 30 min on awake patients with closed eyes (Fig. 2). Signals from the right frontocentral and parieto-occipital leads were pre-amplified (time constant 0.3 sec, low-pass filter 30 c/sec) and recorded on magnetic tape (E. Kaiser's Laboratory, Copenhagen).

Table II. *Intellectual impairment, arterial ammonia concentration, and lactulose (Duphalac®) dosage in patients with liver cirrhosis*

Pat. no.	Degree of intellectual impairment[a]: Without treatment	During treatment	Arterial ammonia concentration (µmol/l)[b]: Without treatment	During treatment	Lactulose dosage (ml × 2)
1	4/coma	3	—	—	60
2	3	3	103 143 102	133/213	40
3	0	0	98 171 96	80	60
4	0	0	84 54 100	93 138	60
5	2	2	83 85	113/126	60
6	3	3	108 146 134	136/123	30[c]
7	3	3	72 120 74	33 77	60
8	2	2	78 7 74	82 74	60
9	4	3	134 71 81	101 135	40
10	3	3	114 181	81 31 164	40
11	Coma	4	—	—	60
Mean			103	108	

[a] 0 = No complaints or signs of intellectual impairment. 1 = Slight complaints or signs of intellectual disturbance with unaltered working capacity. 2 = Grade 1 with reduced working capacity. 3 = Moderate degree of mental disturbance with working incapacity. 4 = Severely disabling mental disturbance, including disorientation.

[b] Blood samples taken at the same time as EEG recordings.

[c] ml × 4.

Both leads gave almost identical results and therefore only those from the parieto-occipital lead are presented.

Period analysis was made of the first 100-sec section of the EEG trace without artefacts on a small, general purpose digital computer (IBM 1800) using a complex of data programs described elsewhere (14). The programs combine wave detection by peak-to-peak analysis and baseline crossings.

The frequency range of 0.5–28.6 c/sec was divided into 21 frequency classes and the percentage activity time was calculated for each class (Figs. 2 and 3).

The dominant frequency was calculated as the mean value of the five consecutive classes with the greatest total percentage activity time. The frequency distributions were compared using Spearman's rank correlation coefficient (r_s) (12). If two frequency distributions are equal r_s = 1.0. Comparisons were made in each of nine patients (nos. 2–10) of EEGs in non-treatment periods, and r_s lay between 0.88 and 0.99, which reflects the spontaneous variation in the EEG spectra.

Criteria for acceptance of change in the EEG related to treatment were that there should be a clear increase in the dominant frequency and that the change in the frequency distribution should be reflected by r_s values below 0.88.

RESULTS

Clinical

Eight patients did not respond to treatment (Table II). Particularly they did not impair following withdrawal of lactulose. Two patients without previous cerebral signs or symptoms of encephalopathy were included only for the EEG studies.

In three patients a clinical improvement was observed within two to five days after the beginning of treatment, and withdrawal of the drug was followed by clinical deterioration (see Case Reports).

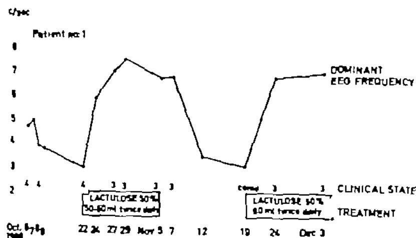


Fig. 1. Relationship between lactulose treatment, clinical state, and the dominant EEG frequency in patient 1.

Fig. 2. EEG before (*I*) and during (*II*) treatment with lactulose, and the EEG spectra (from 100 sec recordings) from all 4 examinations of patient 7. Spectra from the EEG traces shown above are encircled.

Table I *Laboratory data from 11 patients with liver cirrhosis treated with lactulose (Duphalac®)*

Figures within parentheses are normal values

Pat. no	Sex	Age (y)	Months after portacaval operation	Serum bilirubin (mg/100 ml) (<1.3)	P & P time (>65)	Alkaline phosphatases (KA units) (<13)	Serum albumin (g/100 ml) (>3.7)	Serum γ-globulin (g/100 ml) (<1.3)
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6	♀	63	26	3.9	71	39	3 7	1.2
7	♀	66	24	1 4	52	9	4.0	1.9
8	♂	4	36	1.5	66	2	4 2	0.9
9	♀	77	11	1 7	30	11	4.2	1 1
10	♀	64	19	0 6	65	10	3.2	1 1
11	♀	65	4	3.9	64	15	3.7	1.0

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2	3	3	10 4 102	133 213	40
3	0	0	98 1 1 96	80	60
4	0	0	44 54 100	93 138	60
5	2	2	83 85	113 126	60
6	3	3	108 146 134	156/123	30[a]
7	3	3	72 170 74	33 77	60
8	2	2	78 73 4	82/74	60
9	4	3	134 1 81	101/135	40
10	3	3	114/181	81 31/164	40
11	Coma	4	—	—	60
Mean			103	108	

0 = No complaints or signs of intellectual impairment. 1 = Slight complaints or signs of intellectual disturbance with unaltered working capacity 2 Grade 1 with reduced working capacity 3 = Moderate degree of mental disturbance with working incapacity 4 Severely disabling mental disturbance, including disorientation

[b] Blood samples taken at the same time as EEG recordings. ml 4.

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The frequency range of 0.5–28.6 c/sec was divided into 21 frequency classes and the percentage activity time was calculated for each class (Figs. 2 and 3).

The dominant frequency was calculated as the mean value of the five consecutive classes with the greatest total percentage activity time. The frequency distributions were compared using Spearman rank correlation coefficient ( ) (12). If two frequency distributions are equal = 1.0. Comparisons were made in each of nine patients (nos 2 10) of EEGs in non-treatment periods, and lay between 0.88 and 0.99 which reflects a spontaneous variation in the EEG spectra.

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RESULTS

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examination during treatment. One patient under long-term treatment (no. 1) who had had a partial gastrectomy complained of dumping symptoms when the lactulose dose was above 30 ml.

CASE REPORTS

Case 1 (Figs. 1 and 3)

A 56-year-old man precomatous for some months before treatment, unable to read more than newspaper headlines, markedly slow cerebration and partial disorientation. After two days' treatment he could look after himself and after a week he could read books, although obvious signs of intellectual impairment remained. Two days after cessation of treatment he had severe disturbances of consciousness, did not respond when spoken to and had asterixis. One week after resuming treatment his condition and the EEG findings were as during the initial period of treatment and remained stable after 18 months' continued treatment apart from some slight progression of intellectual impairment. He had had two transient periods of confusion associated with sedative drug and alcohol consumption.

Case 9

A 77-year-old woman. Admission to a nursing home thought necessary because of frequent periods of confusion. She complained of fatigue and appeared sluggish. During ten days of treatment she became more awake and active; her memory improved. Nine days after cessation of treatment her condition had returned to the pretreatment level. Treatment was then reinstituted and the response was as before. She has been stable with no episodes of precoma or coma for one year. She lives alone and is able to look after herself.

Case 11

A 65-year-old woman. Lactulose had been given for the preceding four months following portacaval shunt operation. Treatment was interrupted to expose possible indications for continuation. During treatment she required help with personal hygiene, had marked intellectual impairment—was not able to understand or reproduce a printed text, which she could however read. Two days after discontinuation of treatment her consciousness was severely disturbed. She was without reaction to the environment. Five days after resuming treatment her condition was as during the initial treatment, but thereafter deteriorated slowly. For a period lactulose was therefore supplemented with and then substituted by neomycin/bacitracin [illegible] g of each daily per os. There was no improvement and treatment was changed back to lactulose. She died eight months later with progressive hepatic insufficiency and encephalomyelopathy. A post mortem was not performed.

DISCUSSION

After portacaval shunt operations in patients with cirrhosis of the liver the dominant EEG frequency is often found to fall to or below the low[illegible] limit of [illegible] range (8–13 c/sec) (8, 10). This finding is usually ascribed to an increased action [illegible] cerebrotoxic material bypassing the liver through the shunt.

All of the patients in this series had [illegible] portal-systemic shunts, but in none of the p[illegible] with dominant EEG frequencies ab[illegible] [illegible] was any change seen attributabl[illegible] [illegible] treatment. This may indicate that liver detoxif[illegible] [illegible] patients is adequate despite the shun[illegible] [illegible] theless, routine liver function tes[illegible] [illegible] distinguish these patients from those responding to treatment (Table I, $p > 0.1$, Wilcoxon rank sum test).

Three patients with encephalopathy manifested by disturbances of consciousness had dominant EEG frequencies below 7.5 c/sec. In these patients EEG improvement paralleled clinical improvement, but after coma had been relieved, an obvious intellectual impairment persisted despite prolonged lactulose therapy.

Intellectual disturbances are frequent in patients with chronic liver disease and portal-systemic shunts (8, 16), and it has been shown that they are relatively static following shunt operations, which increase the portal-systemic shunting substantially (8). That the intellectual impairment present in most of our patients was unaffected by lactulose supports the assumption that such symptoms are of a chronic, non-toxic nature ("acquired hepato-cerebral degeneration" (15)) and should be distinguished from the disturbances of consciousness ("liver coma"). Such a distinction may explain the otherwise paradoxical finding of Zeegen et al. (17) that lactulose in their series

was beneficial in the three most severely affected of seven patients with encephalopathy.

In two of our patients benefiting from lactulose, withdrawal precipitated hepatic coma. In both patients the dominant EEG frequency was below the normal range even during treatment. Therefore, when lactulose treatment has been found beneficial, any contemplated withdrawal should be most carefully considered.

The side-effects of lactulose seem to be harmless but may be so unpleasant that treatment is refused or must be discontinued. We find this necessary, as do others, in about one in ten patients.

Controlled studies of lactulose are necessary for the evaluation of any possible protective action against the development or progression of the intellectual impairment so often present in patients with liver cirrhosis and portal-systemic shunts (8, 16) and far more common than the recurrent coma syndrome (8).

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HEMODYNAMIC EFFECTS OF OXPRENOLOL ALONE AND COMBINED WITH NITROGLYCERIN IN PATIENTS WITH ISCHEMIC HEART DISEASE

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Abstract. In 16 males with ischemic heart disease the acute hemodynamic effects of a new β-blocker oxprenolol, have been studied during rest and exercise by right heart catheterization technique. The myocardial oxygen-saving effect of oxprenolol was demonstrated by a significant reduction of heart rate during exercise and a widened arterio-venous oxygen difference. Addition of nitroglycerin rapidly and markedly reduced myocardial oxygen demand by reducing the left ventricular filling pressure and the mean systemic arterial pressure. Oxprenolol and nitroglycerin can be combined without any untoward effects and without loss of efficiency of either drug. However no evidence of synergism between the two drugs was found in this study.

Patients with angina pectoris are hemodynamically characterized by impairment of left ventricular function, at least during exercise (19, 24, 29). Congestive heart failure is a serious complication that has been reported after treatment with β-blocking drugs (37) and in most instances this complication has been encountered during treatment with propranolol, by far the most widely used β-blocker (6). A comparison between the acute hemodynamic effects of propranolol and a new β-blocking agent, Ciba 39 089-Ba, i.e. oxprenolol (supplied by Ciba, Sweden), given to patients with predominantly valvular heart disease attracted our interest, since the latter compound produced a similar negative chronotropic effect to that of propranolol but a significantly less marked depression of myocardial contractility (12).

Recently a synergistic effect has been reported after combined therapy with nitrates and β-blockade (propranolol) in patients with angina pectoris (17, 40). The purpose of the present study was therefore to investigate the acute effects of the new β-blocker oxprenolol alone and combined with nitroglycerin, and also to study the effects of nitroglycerin in patients with angina already under the influence of oxprenolol.

SUBJECTS AND METHODS

Sixteen males, between 41 and 62 years of age, all with typical clinical, ECG and coronary angiographic signs of coronary heart disease, were studied. The studies were performed as part of pre- or postoperative evaluation of surgery aimed at improving their myocardial blood supply. This evaluation included a careful medical history, lung function studies (2), hemodynamic evaluation (20), exercise tolerance tests with ECG recordings (16) and coronary angiography (28). None of the patients had signs of congestive heart failure or rheumatic heart disease and all had sinus rhythm at the time of the study.

The studies were made in the morning when the patients were in the postabsorptive basal state. Right heart catheterization was performed with a double lumen catheter and the brachial artery was catheterized using percutaneous technique. Cardiac output was measured by the indicator dilution technique using bromsulphalein (39), injected via a percutaneous polyethylene catheter into the right atrium. Inductance manometers and a 6-channel mingograph (AB Elema-Schönander, Stockholm, Sweden) were used for registration and recording of pressures. The patients were studied in the supine position. Exercise tests were performed on a bicycle ergometer (AB Elema-Schönander, Stockholm, Sweden).

Tensions of oxygen and carbon dioxide and pH in blood samples were measured with conventional electrodes (Instrumentation Laboratories Inc.) and oxygen saturation was calculated from the Severinghaus nomogram (33).

Vascular resistances were calculated according to the following formulae, expressed in arbitrary units.

Pulmonary vascular resistance PVR = $P_P - P_{PCW}/Q$

Systemic arterial resistance SVR = P_{BA}/Q

Left ventricular stroke work was calculated according to the following formula, expressed as gram meters (g.m.)

Table I. *Mean hemodynamic data at rest ($\bar{X}$) in 16 patients with coronary heart disease before (B) and the change 1 hour after (A) i.v. administration of 5 mg oxprenolol*

	HR (beats/min)		SV (ml)		CO (l/min)		A V O_2-diff. (ml/l)		$\bar{P}_{PCV}$ (mmHg)		$\bar{P}_{PA}$ (mmHg)		P_{BA} (mmHg) Syst.		P_{BA} (mmHg) Diast.	
	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A
$\bar{X}$	73.0		87.9		6.3		41 1		9.8		16.3		148.2		83 8	
d		−3.6		−8.3		−0.9		5.2		−0.6		−1.0		−2.4		−2.1
S.D		8.6		14 0		1 4		5.3		5.3		3.3		10.4		11.2
S.E.		2.2		3.5		0.4		1.3		1.3		0.8		2.6		2 8
p		>0.05		<0.05		<0.05		<0.01		>0.05		>0.05		>0.05		>0.05

d = mean difference, S.D = standard deviation, S.E. = standard error, p = probability of the difference, HR = heart rate, SV stroke volume, CO = cardiac output, A V = arterio-venous, $\bar{P}_{PCV}$ = mean pulmonary capillary venous pressure, $\bar{P}_{PA}$ = mean pulmonary artery pressure, P_{BA} = brachial arterial pressure, PVR = pulmonary vascular resistance, SVR = systemic vascular resistance, LVSW = left ventricular stroke work.

$$\mathrm{LVSW} = \frac{SV(\bar{P}_{BA} - \bar{P}_{PCV})\ 13.6}{1\,000}$$

The "rate-pressure product" was calculated as the product of the systolic blood pressure and the heart rate and divided by 100 to reduce it to convenient units (32).

Hemodynamic data were obtained with the patients at rest (measurement 1) and during an exercise test with a level of work predetermined to provoke supportable angina pectoris (measurement 2) (Fig. 1). A few minutes after the exercise study was completed, the tip of the double lumen catheter was withdrawn from the wedge position into the main pulmonary artery. An i. injection of 5 mg oxprenolol was given. The patient was then allowed to rest for approximately 1 hour. The tip of the catheter was then relocated to the wedge position. Rest and exercise studies at the initial work load were now repeated (measurements 3 and 4).

Nine patients who developed angina pectoris during the second exercise period were given 0 5 mg nitroglycerin sublingually and were instructed to continue the exercise under continuous monitoring of the pulmonary capillary venous, pulmonary arterial and brachial arterial pressures. When the discomfort subsided or had completely disappeared, measurements were repeated (measurement 5). This usually occurred within 4 to 6 min after nitroglycerin administration.

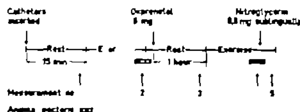


Fig. 1. Design of the study

RESULTS

Measurements obtained *at rest* before and 1 hour after i.v. injection of 5 mg oxprenolol are shown in Table I. Heart rate decreased in most cases (13/16) but the reduction did not attain statistical

Table II. *Mean hemodynamic data during exercise provoking angina pectoris ($\bar{X}$) in 15 patients before (B) and the change 1 hour after (A) i.v. administration of 5 mg oxprenolol*

Abbreviations as in Table I

	HR (beats/min)		SV (ml)		CO (l/min)		A V O_2-diff (ml/l)		$\bar{P}_{PCV}$ (mmHg)		$\bar{P}_{PA}$ (mmHg)		P_{BA} (mmHg) Syst.		P_{BA} (mmHg) Diast.	
	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A
$\bar{X}$	110.3		84.6		9.3		85 7		24.2		32.8		186 6		105 9	
	15		15		15		15		15		15		14		14	
d		−10.3		3.0		−0.5		7.9		−1.6		0.1		−7.8		−7.5
S D.		10 4		16 9		1 4		12 7		6 1		5.9		15 5		15.3
S E.		2.7		4 4		0.4		3.3		1.6		1.5		4 1		4.3
p		<0.01		>0.05		>0 05		<0.05		>0.05		>0 05		>0 05		>0 C

Mean		PVR		SVR		HR BA (syst./100)		LVSW (g.m.)		Pa_{O_2} (mmHg)		Pa_{CO_2} (mmHg)	
B	A	B	A	B	A	B	A	B	A	B	A	B	A
109.8		1.1		17 7		108.9		118.8		83.2		36.4	
	−4 6		0.2		2.4		−8.8		−13 9		−1.6		−0.3
	10 3		0.7		4 8		16.2		19 7		8.9		3.0
	6		0.2		1.2		4.1		4.9		2.2		—
	>0.05		>0.05		>0 05		<0 05		<0 05		>0 05		>0 05

significance ($\bar{d} = -3\ 6$ beats/min) ($p > 0.05$) Stroke volume, cardiac output, left ventricular stroke work and "rate-pressure product" were reduced ($p < 0.05$), while the arterio-venous oxygen difference showed an increase ($\bar{d} = +5$ ml/l) ($p < 0.01$)

Mean pulmonary capillary venous pressure mean pulmonary artery pressure, mean brachial artery pressure, pulmonary vascular resistance and systemic vascular resistance did not change significantly One patient developed angina pectoris at rest and was therefore excluded from exercise studies.

Table II shows recordings made during exercise before and approximately 1 hour after i.v. administration of oxprenolol. This drug reduced heart rate ($\bar{d} = -10$ beats/min) ($p < 0.01$) arterial oxygen tension($\bar{d} = -8$ mmHg) ($p < 0.01$) and "rate-pressure product ($\bar{d} = -9$) ($p < 0.05$) It increased arteriovenous oxygen difference ($\bar{d} = +8$ ml/l) ($p < 0.05$) and pulmonary vascular resistance ($\bar{d} = +0.3$) ($p < 0.01$) Stroke volume, cardiac output, left ventricular stroke work, mean pulmonary artery pressure, mean pulmonary capillary venous pressure mean brachial artery pressure and systemic vascular resistance were unchanged.

Six patients did not develop typical angina pectoris during the second exercise period and were therefore not given nitroglycerin. Addition of nitroglycerin in the remaining nine subjects who developed angina pectoris during exercise under β-blockade (Table III) further reduced arterial oxygen tension ($\bar{d} = -6$ mmHg) ($p < 0.05$). Heart rate, cardiac output, stroke volume left ventricular stroke work, arterio-venous oxygen difference, pulmonary vascular resistance systemic vascular resistance and "rate-pressure product" were unchanged. Nitroglycerin caused, in this situation, a marked reduction of mean

Mean		PVR		SVR		HR BA (syst./100)		LVSW (g.m.)		Pa_{O_2} (mmHg)		Pa_{CO_2} (mmHg)	
B	A	B	A	B	A	B	A	B	A	B	A	B	A
142.6		0 9		16 0		206.0		132.7		88.8		37 1	
14		14		14		14		14		14		14	
	−9.3		0 3		0.2		−26 6		−2 2		−7.9		0.1
	16.8		0 4		3 8		36.2		28 7		8.7		2 5
	4.5		0.1		1.0		9 7		7 7		2.2		0 6
	>0.05		<0 01		>0.05		<0 05		>0 05		<0 01		>0 05

Table III. *Mean hemodynamic data during exercise provoking angina pectoris* ($\bar{X}$) *in 9 patients under β-blockade (5 mg oxprenolol) before (B) and the change after (A) nitroglycerin (0.5 mg sublingually)*

Abbreviations as in Table I

	HR (beats/min)		SV (ml)		CO (l/min)		A V O_2-diff (ml/l)		$\bar{P}_{PCV}$ (mmHg)		$\bar{P}_{PA}$ (mmHg)		P_{BA} (mmHg) Syst.		P_{BA} (mmHg) Diast.	
	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A
$\bar{X}$	99		81		8.0		104		25		34		175		101	
		9		7	7			9		9	9			9		9
$\bar{d}$		−2 4		−1 6		−0.1		−5.8		−16.9		−17.0		−16 0		−2.4
S.D		6.4		11.5		1.0		10 8		5 4		5.5		19.6		16 7
S.E.		2.1		4.4		0 4		3.6		1.8		1.8		6.5		5 6
p		>0.05		>0 05		>0.05		>0 05		<0.001		<0 001		-0 05		>0 05

pulmonary artery pressure ($\bar{d} = -17$ mmHg) ($p < 0.001$) mean pulmonary capillary venous pressure ($\bar{d} = -17$ mmHg) ($p < 0\ 001$) and a slight reduction of the systolic arterial blood pressure ($\bar{d} = -16$ mmHg) ($p < 0.05$) but the mean brachial arterial pressure was not changed.

Table IV shows a comparison of the hemodynamic events during exercise provoking angina pectoris before and after β-blockade in combination with nitroglycerin The combination of nitroglycerin and oxprenolol caused a reduction in arterial oxygen tension ($d = -12$ mmHg) ($p < 01$) but had no influence on heart rate, cardiac output stroke volume, left ventricular stroke work, pulmonary vascular resistance and systemic vascular resistance. There was, however a substantial reduction in mean pulmonary arterial pressure ($d = -17$ mmHg) ($p < 0.001$), mean pulmonary capillary venous pressure ($\bar{d} = -18.6$ mmHg) ($p < 0.001$) and a reduction of the mean brachial arterial pressure ($\bar{d} = -21$ mmHg) ($p < 0.05$) The systolic arterial blood pressure showed a decrease ($\bar{d} = -26$ mmHg) ($p < 0.01$) as did the rate-pressure product ($d = -40$) ($p < 0.01$)

DISCUSSION

Beta-adrenergic blocking drugs were developed for use in clinical medicine on the assumption that a compound which prevented the myocardium from being exposed to the increase in metabolic demand which accompanies release of the neuro-transmitter during stimulation of sympathetic pathways would be useful in the treatment of angina pectoris (26) An important disadvantage of β-blocking drugs, however is depression of the myocardium and recently attention has been directed to the quinidine like myocardial depressant action of propranolol and certain structurally similar drugs (Brit. med. J No. 5743

Table IV *Mean hemodynamic data during exercise provoking angina pectoris* ($\bar{X}$) *in 9 patients before (B) and the change after (A) administration of 5 mg oxprenolol and nitroglycerin (0.5 mg sublingually)*

Abbreviations as in T ble I

	HR (beats/min)		SV (ml)		CO (l/min)		A V O_2-diff (ml/l)		$\bar{P}_{PCV}$ (mmHg)		$\bar{P}_{PA}$ (mmHg)		P_{BA} (mmHg) Syst.		P_{BA} (mmHg) Diast.	
	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A
$\bar{X}$	109		85		9.2		90		26		35		185		113	
		9		7		7		9		9		9		9		9
$\bar{d}$		−7.3		−8 9		−1.3		8.1		−18.6		−17 1		−26.1		−14.3
S.D		9 6		18 0		1.9		8.8		7.3		6.9		19 7		15 3
S E.		3 2		6.8		0 7		2 9		2.4		2.3		6 6		5.1
p		>0 05		>0.05		-0 05		<0 05		<0 001		<0 001		<0 01		<0 05

Mean		PVR		SVR		HR BA (syst./100)		LVSW (g m.)		$P_{a_{O_2}}$ (mmHg)		P_{CO_2} (mmHg)	
B	A	B	A	B	A	B	A	B	A	B	A	B	A
146		0 9		16		200		134		86		39	
9		7		7		9		7		9		9	
	21.1		0.3		0.1		−40 4		−17 7		−11 8		0 3
	21 5		0.7		5.5		30.8		33 1		9 8		1 9
	7.2		0 3		2.1		10.3		12 5		3 3		0 6
	0 05		>0 05		>0.05		<0 01		>0 05		<0 01		>0 05

p. 243 1971). The inhibition of the positive inotropic effects of catecholamines already deprives the heart of an important regulatory mechanism. If this is coupled with a direct cardiodepressant action, there is obviously a far greater danger of congestive heart failure developing (23).

The recently developed compound, Ciba 39 089-Ba, i.e. oxprenolol, is claimed to be free from directly depressing effects on the myocardium and to have a small degree of intrinsic sympathicomimetic activity while retaining the basic properties of β-blocking drugs (38). It is possible that the intrinsic positive inotropic effect of oxprenolol provides a safeguard against the development of cardiac insufficiency (23).

In experiments with isolated human papillary and atrial muscle Nayler et al. (27) found that propranolol exerts a negative inotropic effect in the concentrations needed to block the β-receptors, whereas oxprenolol did not diminish cardiac contractile force until concentrations about 10 times greater were used. In reserpinized cats the direct cardiac action of oxprenolol consists of a slight positive inotropic and chronotropic effect which propranolol lacks (23). This ef e t of o prenolol is qualitatively similar to that reported previously for pronethalol (3). In estigators on modified Sarnoff Berglund left entricular work preparations and isolated papillary muscle studies have shown that the depressant effect of propranolol on left ventricular function is more marked than that caused by an equipotent β-blocking dose of oxprenolol (25).

The effects of β-adrenergic blocking drugs may be either beneficial or harmful to patients with angina pectoris (11). Beneficial effects may result from a reduction in myocardial oxygen requirements brought about by a decrease in heart rate (30) a decrease in the velocity and the extent of myocardial fibre shortening (35) and reduction in arterial pressure (10). A number of studies have also demonstrated a reduction in myocardial oxygen consumption following β-blockade (22, 41).

Mean		PVR		SVR		HR BA (syst /100)		LVSW (g m)		$P_{a_{O_2}}$ (mmHg)		$P_{a_{CO_2}}$ (mmHg)	
B	A	B	A	B	A	B	A	B	A	B	A	B	A
133		1.4		17		172		120		80		39	
9		7		7		9		7		9		9	
	10 0		−0 1		−1 0		−12 3		4 9		−6 0		0 2
	16 2		0 7		3 5		21.8		31 3		5.7		2 1
	5 4		0.3		1.3		7.3		11 9		1.9		0.7
	0 05		>0 05		>0 05		>0 05		>0 05		<0 05		>0 05

Harmful effects from β-blockade might occur from an increase in cardiac dimensions (5) which, according to the law of Laplace, will produce an increase of the ventricular wall tension under any given pressure. Furthermore, prolongation of the systolic ejection period has been observed, which will extend the time during which the ventricles must maintain tension and thereby increase myocardial oxygen demand (14 30).

Myocardial oxygen consumption could not be assessed directly in the present study It is, how ever known that β-blockade decreases myocardial oxygen consumption not only by a diminished heart rate but also by a rate-independent reduction of the velocity of myocardial contraction (4 7)

Oxprenolol alone or in combination with nitroglycerin failed to cause a significant slowing of the heart rate at rest, and only a slight reduction of the cardiac output and of the stroke volume was observed (Table I) In normal subjects similar effects have been described by Ulrych and Chrpová (38). A more pronounced negative chronotropic effect at rest, with no change in cardiac output or stroke volume has been reported in patients with angina pectoris by Sharma et al. (34) and in patients with predominantly valvular heart disease by Grandjean and Rivier (12).

During supine leg exercise somewhat more pronounced changes were observed (Table II) The reduction in heart rate ($\bar{d} = -10$ beats/min) ($p < 0.01$) was partly offset by a not significant increase in stroke volume ($\bar{d} = 3$ ml) ($p > 0.05$) resulting in a decrease of the cardiac output ($\bar{d} = -0.5$ l/min) ($p > 0.05$). Similar results after oxprenolol administered i.v have been obtained by Sharma et al. (34) and by Grandjean and Rivier (12). The increase in arterio-venous oxygen difference noticed after oxprenolol should probably be regarded as a result of a drug-induced hypokinetic circulation. Simultaneously a significant fall in the arterial oxygen tension was observed This was not caused by diminished alveolar ventilation, as P CO_2 remained unchanged. Adrenergic β-blockade is known to increase airway resistance (18) This will probably cause an uneven ventilation of the lungs. Ventilation-perfusion disturbances may thus account for the observed reduction in the arterial oxygen tension.

Oxprenolol did not affect the pressures in the lesser circulation at rest or during exercise. A significant increase in the pulmonary vascular resistance during exercise was observed in this study and also by Sharma et al. (34) as a result of diminished cardiac output with unchanged pressure gradient over the pulmonary vascular bed. The systemic vascular pressures were some what lower especially during exercise, but the differences did not attain statistical significance, and the systemic vascular resistance remained unchanged after drug administration.

In the present investigation no change in left ventricular stroke work was observed after oxprenolol with or without nitroglycerin. Left ventricular stroke work cannot be considered an adequate measure of myocardial oxygen consumption, as changes in cardiac volume and contractility are known to occur with both β-blockade and nitroglycerin (5 8 21 35 36, 41). However in a thorough analysis of different indices of left ventricular work no measurable change after β-blockade was found even when changes in ventricular volume and duration of systole were included in the assessment (13)

The myocardial oxygen-saving effect of oxprenolol was, in the present study thus demonstrated only by a significant reduction of heart rate during exercise and a widened arterio-venous oxygen difference suggesting decreased cardiac work and output. These changes were however not accompanied by any harmful negative inotropic effects of the drug.

The product of the heart rate and the systolic arterial pressure has recently been employed as an index of cardiac work as opposed to external work (32) In individual patients, although the external work load required to produce angina varied, Robinson (32) found that the "rate-pressure product" at which angina occurred was remarkably constant. Oxprenolol in this study caused a small but significant decrease in the rate-pressure product" at identical levels of exercise. The "rate pressure product" is, however valid for predicting neither the critical level of cardiac work nor myocardial oxygen consumption when changes in heart size and duration of ejection time are not taken into account (9 15, 32)

The hemodynamic changes occurring with exercise-induced angina, and the modification of these abnormalities following the administration of

nitroglycerin, were described originally by Müller and Rörvik (24). Typical angina is accompanied by elevation of pulmonary capillary wedge and artery pressures, changes suggestive of left ventricular failure (19). Following the administration of nitroglycerin sublingually these pressures return to normal without reduction in cardiac work, indicating a shift in the ventricular function curve to the left with equal amount of work performed at lower filling pressures. The peripheral pooling of blood with reduced venous return to the heart will result in smaller cardiac size with diminished myocardial oxygen demand and in reduced ventricular wall tension with facilitated coronary perfusion (1, 29).

Beta-blockade and nitroglycerin act through different mechanisms in diminishing the needs of the heart for oxygen (21) and some authors have claimed not only an additional effect of β-blockade and nitroglycerin but also a synergistic one (9, 17, 40).

Statistical comparisons (t-test) were made between the hemodynamic data from this group of patients and those from a group with IHD previously investigated in this laboratory (1). The observed difference between means and standard deviations in the two groups during angina pectoris was not statistically significant. This allows for a comparison of the response to nitroglycerin accompanied by oxprenolol as seen in the present study with the response to nitroglycerin alone, the condition which prevailed in the previously studied group. The acute hemodynamic effects of nitroglycerin were alike in both groups whether or not oxprenolol was previously administered. However when the effects produced by the combination of oxprenolol and nitroglycerin were compared statistically with changes brought about by nitroglycerin alone it was noted that heart rate and cardiac output were significantly lower and that the arterio-venous oxygen difference was significantly higher among the patients who had been given both drugs ($p < 0.01$). This suggests that the combination of oxprenolol and nitroglycerin is more favourable than nitroglycerin alone for patients with angina pectoris. The two types of therapy can be combined without any untoward acute effects and without loss of efficiency of either drug. No evidence was, however found of synergism in the sense that hemodynamic effects of the two drugs administered together should be greater than the sum of their separate effects.

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ANGIOTENSIN II PLASMA LEVELS IN HYPERTENSIVE PATIENTS

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Abstract. The response of peripheral venous plasma angiotensin II (A-II) to upright posture combined with an injection of furosemide was higher in patients with hypertension and associated renal parenchymal disease, renal artery stenosis or malignant hypertension than in patients with essential hypertension and normotensive control patients. Very low responses to the same stimuli were observed in primary aldosteronism. Markedly increased A-II levels were measured in the renal veins of the affected kidneys in hypertensive patients with associated unilateral renal parenchymal disease or renal artery stenosis, suggesting that human kidneys contain considerable converting enzyme activity. Plasma A-II levels were normalized in three patients with primary aldosteronism after removal of an adrenal adenoma and in one patient with malignant hypertension after bilateral nephrectomy and renal transplantation.

Most of the biological activity of the renin angiotensin system is mediated by angiotensin II (A II). Thus, it appears a valuable index of the activity of this system to determine circulating plasma or blood concentrations of A II.

High basal plasma or blood A II levels have been reported in malignant hypertension, hypertension with associated renal parenchymal disease or renal artery stenosis (2, 5, 6, 7, 9, 10, 11). However, the possible role of A II and its congeners in human hypertension is still an enigma. Increased plasma A II levels have also been reported in non-hypertensive diseases like liver cirrhosis with ascites, and in nephrotic syndrome (5, 6, 7, 9, 10, 11, 20). Both high and low plasma A II levels have been observed in essential hypertension and low A-II levels are found in primary aldosteronism (2, 5, 6, 7, 9, 10, 11).

High renal venous A II levels have been observed in the renal vein of the affected kidney in hypertensive patients with associated renal artery stenosis or unilateral parenchymal disease (11, 19).

In healthy individuals upright posture causes a smaller rise in plasma A II levels than does the acute administration of diuretics (7). The present report extends previously published works by presenting data on plasma A-II levels in response to upright posture and an injection of furosemide in various forms of hypertension. Data on separate measurement of A II concentration in the renal veins of hypertensive patients are also presented.

PATIENTS AND METHODS

Assay

Radioimmunoassay of A-II was performed as previously described (10). Venous blood collected into syringes containing EDTA and BAL was immediately chilled to 0°C, centrifuged in cold, and the plasma was kept frozen at −20°C until extraction of A-II with Fuller's earth. 5-val-angiotensin-II-amide (Hypertensin, Ciba) was used as standard for iodination with [125]I (Amersham) and coupled to human serum albumin (Kabi, Sweden) with carbodiimide[18] for immunization of rabbits. Antiserum diluted 1:10 000–1:30 000 was used in the assay and bound tracer hormone was separated with coated charcoal. The antiserum used showed the following crossreactions with unlabelled peptides (relative potency of inhibition): ileu-5-angiotensin-I < 0.5%, 5-ileu heptapeptide 7%, 5-val-hexapeptide (3–8) 4%. The recovery of 50–2 000 pg of synthetic A-II added to pooled plasma was 82 ± 14%. The limit of sensitivity was 1.5 pg/ml of immuno-reactive A-II.

Hypertensive patients

Routine examinations. The following examinations were performed: serum creatinine, sodium, potassium, calcium, chloride, microscopy of urine, urinary protein and excretion of metanephrines, adrenaline, vanillinemandelic acid, 5-hydroxyindoleacetic acid, 17-ketosteroids, ketogenic steroids, and aldosterone. Chest X-ray, pyelography and nephrography were performed, and renal arteriography was carried out when pyelography or nephrography revealed suspect changes.

Essential hypertension. The 13 patients belonging to this group had diastolic blood pressure repeatedly above 100 mmHg. They had also undergone renal needle biopsy

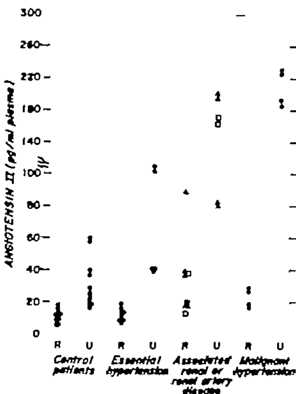


Fig. 1. A-II levels in peripheral venous plasma of patients with hypertension compared with normotensive control patients. Note the change in scale for values above 100 pg/ml. R = recumbency U = upright posture + furosemide; Δ = unilateral, ▲ = bilateral, parenchymal renal disease – unilateral, ■ = bilateral renal artery stenosis.

Hypertension with associated renal parenchymal disease or renal artery stenosis. Associated unilateral parenchymal renal disease with clearly diminished excretory functions of the involved kidney was observed in 5 patients. Three patients had bilateral parenchymal disease of the kidneys and renal insufficiency. Unilateral renal artery stenosis with post-stenotic dilatation of the artery and diminished excretory capacity of the involved kidney was observed in 4 patients. One patient had multiple bilateral renal artery stenoses.

Malignant hypertension. Accelerated hypertension with IV eyeground lesions (Keith-Wagener) and diastolic blood pressure repeatedly above 120 mmHg was found in 7 patients, 4 of whom had proteinuria with slight renal insufficiency (serum creatinine 1.8–3.1 mg/100 ml).

Primary aldosteronism. Five patients had primary aldosteronism confirmed by postoperative microscopy. Of these patients 3 had solitary adrenocortical adenoma and hyperplastic adrenal cortex with hyperplastic micronoduli.

Drugs and diet

Diuretics and antihypertensive drugs were withdrawn for 1 week before collection of blood samples. Contraceptives were withdrawn 2 months previously. Blood samples were drawn after at least 5 days in the hospital on about 80 mEq sodium per day regular hospital diet.

Collection of blood samples. Peripheral venous blood was drawn at 8 a.m. after one night's recumbency and fasting for 12 hours. Thereafter the patient slowly walked for 2 hours, and 20 mg furosemide was given i.m. at 9 a.m. A second sample was drawn at 10 a.m.

Renal venous blood was drawn by the Seldinger technique under X-ray television control after a premedication of meperidine hydrochloride 50 mg, and promethazine hydrochloride 25 mg, both i.m.

RESULTS

Control group

The median value of peripheral venous plasma A II in normotensive control patients was 12 pg/ml at recumbency (range 6–34) and 25 pg/ml (range 17–60) after upright posture and furosemide injection (Fig. 1). The plasma A II response to the combined stimulus was significant ($p < 0.01$).

Essential hypertension

In benign essential hypertension (Fig. 1) peripheral venous plasma A II was not significantly higher than in the control group at recumbency, the median value being 13 pg/ml (range 3–52). However, after upright posture and an injection of furosemide the peripheral plasma A-II levels were higher than the corresponding values in the control group (median 48 pg/ml, range 18–230, $p < 0.05$). In essential hypertension there was a significant rise of plasma A II levels in response to upright posture and furosemide ($p < 0.01$), though some patients responded poorly to these stimuli.

Hypertension and associated renal parenchymal or renal artery diseases

In patients with hypertension and unilateral renal parenchymal disease (Fig. 1) the peripheral venous plasma A-II values at recumbency (median 39 pg/ml, range 18–89) and after upright posture and furosemide (median 194 pg/ml, range 82–217) were higher than the corresponding values in essential hypertension ($p < 0.05$). However, a considerable overlap was observed. High responses of peripheral venous plasma A II levels to upright posture and furosemide were also observed in patients with unilateral or bilateral renal artery stenosis (Fig. 1). In 3 patients with bilateral

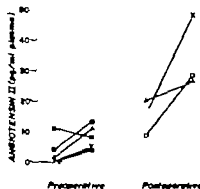


Fig. 2. A-II levels in peripheral venous plasma of patients with primary aldosteronism. R = recumbency U = upright posture + furosemide. Three patients with solitary adrenal cortical adenoma had "normal" A-II levels 3-8 months after surgical removal f the tumor. Two patients (□,) improved, while the third (Δ) remained hypertensive.

parenchymal renal disease renal insufficiency and hypertension (Fig. 1) rather high plasma A-II levels were observed at recumbency and a moderately high response was seen to upright posture and furosemide.

Malignant hypertension

The patients with malignant hypertension (Fig. 1) all had high peripheral venous plasma A-II levels in response to upright posture and furosemide (median 194 pg/ml, range 117–295) clearly higher than in the patients with benign essential hypertension ($p < 0.01$) However at recumbency several plasma A-II values in the "normal" range were observed (median 28 pg/ml, range 17–84).

Primary aldosteronism

In all patients with primary aldosteronism (Fig 2) ery low peripheral venous plasma A-II levels were observed, some of them being undetectably low (< 1.5 pg/ml) at recumbency Slight increases in plasma A II levels were observed in 4 out of 5 patients after upright posture and furosemide. However this response of peripheral plasma A-II levels to the combined stimulus was smaller than in any other hypertensive patient investigated. In 3 patients with a solitary adrenocortical adenoma plasma A II levels were normalized postoperatively (Fig. 2). Postoperativ A II measurements could not be carried out in the remaining patients with hyperplastic micronoduli of the adrenal cortex.

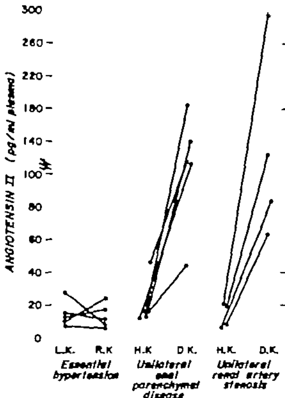


Fig 3 A-II levels in the renal veins of patients ith unilateral parenchymal renal disease and unilateral renal artery stenosis compared with patients ith essential hypertension. Note the change in scale for values above 100 pg/ml. L. K. = left kidney R. K. = right kidney H K. = healthy kidney D K. = diseased kidney

Determination of renal venous A-II

Elevated plasma A-II levels were consistently found in the renal vein of the affected kidney of hypertensive patients with either unilateral renal parenchymal disease or renal artery stenosis (Fig. 3) whereas the patients with essential hypertension and symmetrical excretory functions of the kidneys had almost equal and fairly low plasma A II values in both renal veins.

Effect of renal transplantation

One patient with malignant hypertension, severe renal insufficiency and a high response of peripheral venous plasma A II to upright posture and furosemide (Fig. 4) had undetectable plasma A-II levels after bilateral nephrectomy after

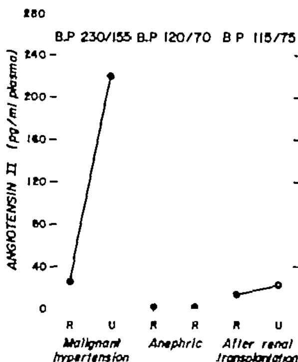


Fig 4 A II levels in peripheral venous plasma of a patient with malignant hypertension, before and after bilateral nephrectomy and renal transplantation. R = recumbency U = upright posture + furosemide B. P = blood pressure (mmHg)

ich she became normotensive. Six weeks after essful renal transplantation her peripheral ous plasma A II values were within "normal" limits, and she was still normotensive

DISCUSSION

The present results of peripheral venous plasma A II measurement suggest that a combined stimulus of upright posture and the injection of furosemide, a potent stimulator of renin secretion, reveals differences in activity of the renin-angiotensin system where a single determination of the plasma A II levels would fail to do so. The response of plasma A II levels to various stimuli may be more useful than measuring only the "basal" plasma A II levels at a fixed time posture, diet, and state of electrolyte and water balance.

All the patients with primary aldosteronism had low or undetectable plasma A II levels. Four out of five responded with a low rise in plasma A II levels to upright posture and furosemide This increase was smaller than in any other hypertensive patient investigated a fact of clinical usefulness.

The observation of raised "basal" plasma A II levels in hypertension associated with renal parenchymal disease or renal artery stenosis, and in malignant hypertension has been previously reported (2, 5 6, 7 9 10, 11). Our observation of particularly high responses of plasma A II levels to upright posture and furosemide injection in such hypertensive patients may be of diagnostic significance.

Although we observed consistently elevated A II plasma levels in hypertensive patients with associated renal parenchymal disease, renal artery stenosis, and in malignant hypertension as previously reported by others (2, 5 6, 7 9 10 11) and from our laboratory (10), we would hesitate to consider this as evidence that A-II is in any way involved in the development and maintenance of hypertension in these patients. High circulating plasma A II levels may equally well be secondary to increased activity of the sympathetic nervous system renal damage or both (21) In support of this we have observed decreases in plasma A II levels in patients treated for malignant hypertension with β-adrenergic blocking drugs (unpublished) as has been recently reported also by others (7). On the other hand we have also observed high plasma A II levels in patients with thyrotoxicosis (unpublished), perhaps due to the *increase of sympathetic nervous system activity* occurring in these patients.

Recent reports on the failure to affect experimentally induced renal hypertension in rabbits by immunization against A II (8 15) have raised the question whether A II has any role in the development of renal hypertension. On the other hand many recent reports suggest several possible mechanisms whereby A II and its congeners could affect the systemic blood pressure, e.g. by causing release of catecholamines from the adrenal medulla (17) by stimulating the adrenal cortex (1), by increasing the uptake of and reactivity to catecholamines in blood vessels (14), by causing release of antidiuretic hormone (16), or by directly stimulating the cardiovascular centre in the brain (18).

The high levels of plasma A II in the renal vein of the affected kidney in hypertension associated with unilateral renal parenchymal dis-

ease or renal artery stenosis (Fig. 3) confirm previous reports (11 19) and suggest that human kidneys contain considerable converting enzyme activity. Whether renal converting enzyme is of importance in these patients seems to be worth further investigation. Simultaneous measurement of renal venous renin, angiotensin I and II would appear useful to throw light upon this question.

The antiserum used in this assay showed a low degree of crossreaction with A II fragments likely to interfere with the immunoassay of plasma A II (3). Since we used venous blood for A II determination, which is a good approximation to arterial plasma A II levels (4 7), many of our patients actually had extremely high arterial plasma A-II responses to upright posture and furosemide if we assume the true arterio-venous difference of A-II to be close to 80% (4).

The present results bring further evidence that A-II measurement is useful in estimating the activity of the renin-angiotensin system. However a relevant interpretation of the estimated A-II values may frequently be impossible without a knowledge of the state of angiotensin receptors (12) and their possible inhibitors and cofactors. More information about the events at the receptor level would make it easier to understand primary and secondary phenomena in high blood pressure.

ACKNOWLEDGEMENTS

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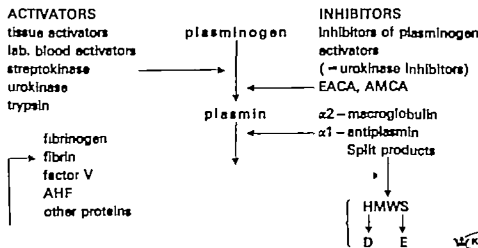
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Urinary tract haemorrhages may be caused by increased fibrinolytic activity Cyklokapron reduces or arrests fibrinolytic bleeding

In recent years fibrinolytic inhibitors have found wide spread use in a number of haemorrhagic conditions, particularly in urinary tract haemorrhages and in connection with prostate surgery. Urine contains urokinase. This enzyme activates the conversion of the plasminogen present in the blood and blood clots into the proteolytic enzyme plasmin, which dissolves clots and thus sustains various types of haemorrhage in the urinary tract. Cyklokapron produces a haemostatic effect by counter acting the activity of urokinase.

The Swedish investigators, Lennart Andersson and Inga Marie Nilsson, have obtained good clinical results by administering Cyklokapron to patients suffering from haemorrhages in the upper and lower urinary tract as well as postoperative bleeding following prostate surgery. Patients suffering from haematuria as a result of general fibrinolysis were also included in the investigation. Bleed ng ceased completely in all the patients in the latter group, as was the case with most of the other patients.

the fibrinolytic system



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FATTY ACID COMPOSITION OF SERUM LIPIDS IN MEN WITH MYOCARDIAL INFARCTION

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Abstract. Studies of the fatty acid composition in cholesterol esters, phospholipids and triglycerides in serum have been made in 73 men with acute myocardial infarction (AMI) and in 32 healthy men of comparable age. Of the patients 36 had previously had myocardial infarction or history of angina pectoris, while for the other 37 the present attack was their first symptom of coronary heart disease (CHD). Significant differences in fatty acid composition were observed between the two groups of patients with higher percentages f linoleic acid in patients with old CHD. Since linoleic acid proportions in serum to large extent reflect dietary habits, it is thought that patients with an old CHD have been more assiduous in following diet recommendations given to the hole population, advocating cholesterol-lowering diets" than subjects with no history of cardiac disease. It is concluded that data concerning fatty acid composition in patients with history of old CHD cannot be used to evaluate dietary habits prior to the manifestation of CHD. It was demonstrated that in patients with an AMI as the first manifestation of CHD linoleic acid percentages were lower than in controls. The finding indicates dietary habits in the patients characterized by high intake of saturated and monounsaturated fats, low intake of essential fatty acids, and possibly high intake of simple sugars. Differences were also observed in pentaenoic and hexaenoic acids, with higher values in the patient group. This finding does not support the assumption that pentaenoic and hexaenoic acids are significant in the prevention of CHD. In smaller number of patients, admitted to the hospital within 6 hours after the initial attack of pain, studies were made on admission and on the following morning. These studies gave no indication f important changes in fatty acid composition occurring during the first 24 hours of the disease and since all studies reported here were performed within 24 hours after onset of the attack, the results are probably representative of preinfarction fatty acid composition.

There is considerable evidence connecting atherosclerosis to dietary fats and blood lipids. It is generally believed that the usual diet of populations in Western countries—high in saturated fats and simple sugars—has pathogenetic importance for and is at least partly responsible for the high incidence of coronary heart disease (CHD) Epidemiologic studies have demonstrated the difference between these populations and populations with lower socioeconomic standards, both in blood lipids and incidence of CHD (18) Large prospective studies have shown that the risk of CHD is proportional to antecedent serum cholesterol (17 39). Since serum cholesterol in man can be changed by altering the kind and amount of dietary fat (1 2, 5), it is possible that differences in quality and quantity of fats are contributory factors in explaining individual predispositions to CHD Trials with "cholesterol-reducing" diets have demonstrated a beneficial effect on morbidity of CHD (8 30, 38), but it remains uncertain whether or not differences in diet have actually existed between CHD patients and the healthy part of the population.

It has been suggested that determination of the fatty acid composition of serum lipids may be used as a clue to the composition of dietary fat (11 13 28). The essential fatty acid, linoleic acid, is not synthesized to any important degree in the body and several studies have demonstrated the dependence of the proportion of linoleic acid in serum upon the linoleic acid intake in food. Experiments ha e been made in rats (24), dogs (34), rabbits (37), pigs (29) monkeys (10), and chickens (6), showing the variation of linoleic acid percentages in blood with the intake of food. The influence of dietary fat upon the fatty acid composition of the various lipid classes is also very pronounced in man (3 11 13 15 21 28,

Table I. *Serum lipids in 12 patients admitted less than 6 hours after onset of myocardial infarction (mean mg/100 ml)*

ns = not significant

	On admission	Next morning	Statistical evaluation
Total cholesterol	298	288	ns
Cholesterol esters	205	203	ns
Phospholipids	273	260	ns
Triglycerides	96	94	ns

35). Changes occur fairly rapidly and after 7 weeks on a particular diet stable patterns are obtained (3).

Several attempts have been made to apply the gas-chromatographic analysis of serum lipids in the study of CHD patients, but results of different studies have been variable.

With 1. and 18 subjects, respectively, James et al. (16) and Lawrie et al. (27) studied patients with previous myocardial infarction or with myocardial ischaemia, but found no disturbances in linoleic acid percentages when compared with healthy subjects. More recently Bang et al. (4) studied a material of 21 male and 11 female patients with myocardial infarction within 72 hours after the onset of the acute attack. The survivors were re-examined 4–12 months later. The fatty acid composition of serum lipids in these patients was not significantly different from that of a control material.

Schrade et al. (36) examined a group of hyperlipemic atherosclerotic patients (previous myocardial infarction, angina pectoris, or claudicatio intermittens) and 17 patients with idiopathic hyperlipemia without symptoms of atherosclerosis, and demonstrated lower linoleic acid values in cholesterol esters and phospholipids for both hyperlipemic groups than for controls. Böttscher et al. (7) studied 4 men with severe aortic atherosclerosis (aged 41 to 57 years) compared with 4 healthy men (aged 41 to 57 years) and found low linoleic acid proportions in cholesterol esters of the patients. The author could not exclude the possibility that the difference between patients and controls was wholly or in part due to age differences.

A more prospective approach to the problem has been made by Kingsbury et al. (19), who examined 146 male patients with claudication due to atherosclerosis. Re-evaluation of the material 3–4 years later showed that the development of non-fatal and fatal attacks of myocardial infarction was significantly associated with reduced concentrations of initial dienoic acid values (alkali isomerization method) in plasma cholesterol esters.

From the publications cited it is evident that results regarding fatty acid composition in patients with CHD have been equivocal. The variations in results may be caused partly by differences in conditions under which the studies were made. Several factors may invalidate the results of such studies. Thus, the fatty acid composition of serum lipids has been demonstrated as undergoing alterations in the days following the onset of myocardial infarction (22), and values obtained in this phase of the disease may therefore not be representative of the pre-infarction values. Studies made in a later phase after recovery from acute myocardial infarction (AMI), or in patients with angina pectoris, are probably invalidated by the general health propaganda in mass media, advocating a diet high in polyunsaturated fatty acids. From studies of the fatty acid composition in adipose tissue there are indications that patients with angina pectoris or with previous myocardial infarction have a greater impetus to react to these recommendations than the general population (23).

Large scale prospective studies concerning fatty acid composition of serum, comparable to those made for serum cholesterol (17, 39), would probably help to elucidate the problem but would prove costly due to the time-consuming procedures involved in the methods for separation and gas-chromatographic investigation of the serum lipid fractions.

The aim of the present investigation was to evaluate dietary habits of patients with AMI by means of a study of the fatty acid composition of serum lipid fractions. Attempts were made to take into consideration the objections against such studies mentioned above, by performing all lipid determinations within 24 hours following the onset of the disease, and by strictly separating patients with and without histories of previous CHD.

In a smaller group of patients admitted to the hospital not later than 6 hours after the initial attack of pain, determinations were made on ad-

Table II. *Fatty acid composition of serum lipids in 12 patients admitted less than 6 hours after onset of myocardial infarction (mean %)*

ns = not significant

Fatty acids	Cholesterol esters On admission	Cholesterol esters Next morning	Cholesterol esters Statistical evaluation	Phospholipids On admission	Phospholipids Next morning	Phospholipids Statistical evaluation	Triglycerides On admission	Triglycerides Next morning	Triglycerides Statistical evaluation
Myristic							1 2	1.0	ns
Palmitic	9.9	9.8	ns	25.8	25.6	ns	20.5	21 0	ns
Palmitoleic	3.7	3.6	ns	1.4	1.3	ns	4.9	5.4	ns
Stearic	0.7	0 8	ns	12 0	11.8	ns	5.8	5 4	ns
Oleic	20.2	19.8	ns	11 1	10.8	ns	40 6	41.3	ns
Linoleic	53.5	54 0	ns	20.9	19.9	ns	17 4	17.1	ns
Eicosatrienoic				2.4	2.4	ns			
Arachidonic	5.4	5 6	ns	6.0	7.2	$p < 0\ 05$	1.4	1.0	ns
Behenic				2.6	2.5	ns			
Eicosapentae-noic	3 4	3.6	ns	3.7	3 7	ns			
Docosapentae-noic				2.7	2.4	ns			
Docosahexae-noic				6.1	6.7	ns	2 0	2.1	ns

mission as well as on the following morning to learn what changes, if any occur in this early phase.

MATERIAL AND METHODS

The patients with AMI comprised 73 men admitted to the hospital in the years 1968–69. They were selected from the age group 40–70 years, and only patients, from whom blood could be drawn in the fasting condition less than 24 hours (mean 17 hours) after the onset of the initial pain, were included. Diagnosis of myocardial infarction was made according to standard criteria with the characteristic pain, ECG changes, S-GOT elevation to 50 units or more, and elevation of ESR, WBC and temperature. Patients known to have diabetes mellitus, hypothyroidism, or renal disease, as well as patients showing glucosuria on admission, were excluded from the study.

Of the patients participating in the study 36 had previously experienced myocardial infarction or had history of angina pectoris (mean age 59 1 years). No patient in this group had received specific dietary instructions or been under any dietary control prior to the current admission to hospital.

For the remaining 37 the present attack was their first symptom of CHD (mean age 58.8 years).

The two groups have been treated separately in the calculations of results and in the Tables.

A control material included 32 healthy men, office employees and workers of an industrial factory. They were selected from the same age group as the patients (mean age 58.1 years). Each passed through a thorough investigation including physical examination, BP reading, examination of the urine, determination of Hb and ESR, and 12-lead ECG, without important findings. Subjects with previous severe disease, history in any way suggestive of cardiac disease, any disease known to affect lipid metabolism or recent illness of any kind, were excluded, as were they who had undergone recent weight changes. All claimed to consume an "ordinary" diet.

The methods used in the determination of serum cholesterol, phospholipids, triglycerides, and for their fatty

Table III. *Concentrations of serum lipids in men with myocardial infarction and controls (mg/100 ml)*

Mean with S.D within parentheses, ns = not significant

	A. Without previous CHD ($N = 37$)	B. With previous CHD ($N = 36$)	C. Controls ($N = 32$)	Statistical evaluation AB	Statistical evaluation BC	Statistical evaluation AC
Total cholesterol	274 (39)	252 (69)	261 (41)	ns	ns	ns
Cholesterol esters	201 (28)	191 (49)	199 (34)	ns	ns	ns
Phospholipids	264 (30)	254 (49)	262 (33)	ns	ns	ns
Triglycerides	87 (38)	91 (36)	94 (50)	ns	ns	ns

Table IV *Fatty acid composition of serum lipids in men with myocardial infarction and controls* (%)

Mean with S.D. within parentheses; ns = not significant

	A. Without previous CHD (N = 37)	B. With previous CHD (N = 36)	C. Controls (N = 33)	Statistical evaluation AB	BC	AC
Cholesterol ester fatty acids						
Palmitic	11.5 (2.0)	10.8 (1.6)	9.2 (1.5)	ns	$p<0.001$	$p<0.001$
Palmitoleic	4.9 (1.8)	3.8 (1.2)	3.3 (0.8)	$p<0.01$	ns	$p<0.001$
Stearic	1.2 (0.5)	1.2 (0.5)	1.0 (0.3)	ns	ns	ns
Oleic	22.6 (4.0)	19.9 (3.0)	19.6 (2.6)	$p<0.01$	ns	$p<0.001$
Linoleic	47.1 (7.9)	52.7 (6.0)	54.9 (4.6)	$p<0.01$	$p<0.05$	$p<0.001$
Arachidonic	5.1 (1.4)	4.7 (1.5)	4.9 (0.9)	ns	ns	ns
Eicosapentaenoic	2.9 (2.2)	2.5 (1.7)	1.8 (1.1)	ns	ns	$p<0.001$
Phospholipid fatty acids						
Palmitic	26.7 (2.4)	25.8 (2.8)	24.7 (2.8)	ns	ns	$p<0.01$
Palmitoleic	1.7 (0.5)	1.8 (0.5)	1.7 (0.6)	ns	ns	ns
Stearic	12.9 (1.2)	13.2 (1.7)	13.5 (1.4)	ns	ns	ns
Oleic	12.7 (1.7)	11.9 (1.2)	12.3 (1.7)	$p<0.05$	ns	ns
Linoleic	17.0 (3.8)	19.4 (3.3)	22.4 (2.8)	$p<0.05$	$p<0.001$	$p<0.001$
Eicosatrienoic	2.4 (0.7)	2.5 (0.8)	2.4 (0.6)	ns	ns	ns
Arachidonic	7.1 (1.2)	6.8 (1.4)	6.4 (1.4)	ns	ns	$p<0.05$
Behenic	2.4 (1.0)	2.4 (0.9)	2.4 (0.8)	ns	ns	ns
Eicosapentaenoic	*3.5 (2.0)*	*3.1 (1.1)*	*.6 (1.3)*	ns	ns	$p<0.05$
Docosapentaenoic	3.3 (1.0)	3.1 (0.6)	2.8 (1.1)	ns	ns	$p<0.05$
Docosahexaenoic	5.2 (1.8)	5.1 (2.4)	3.3 (1.1)	ns	$p<0.001$	$p<0.001$
Triglyceride fatty acids						
Myristic	1.8 (0.7)	1.4 (0.6)	2.2 (1.1)	ns	ns	ns
Palmitic	24.5 (2.7)	23.1 (2.6)	22.1 (3.2)	$p<0.05$	ns	$p<0.01$
almitoleic	6.3 (1.0)	5.5 (1.0)	5.2 (0.9)	$p<0.01$	ns	$p<0.001$
	3.6 (1.1)	3.4 (1.2)	3.5 (1.2)	ns	ns	ns
Oleic	40.2 (3.4)	39.5 (3.8)	39.6 (4.2)	ns	ns	ns
Linoleic	11.8 (3.6)	15.4 (4.1)	14.5 (3.6)	$p<0.001$	ns	$p<0.01$
Linolenic	0.6 (0.3)	0.8 (0.4)	1.2 (0.4)	ns	ns	ns
Arachidonic	1.0 (0.5)	1.0 (0.4)	1.1 (0.4)	ns	ns	ns
Docosahexaenoic	1.4 (1.3)	1.7 (1.2)	1.1 (0.8)	ns	ns	ns

acid composition, have been described elsewhere (20, 21).

Blood for the lipid analyses in the three groups of participants was drawn in the morning following 12 hour fast.

In 12 patients from the two groups, admitted to the hospital within 6 hours after the onset as defined by the initial attack of pain, blood was drawn on admission (mean 4 hours after onset) and on the following morning (mean 20 hours after onset).

RESULTS

Table I shows concentrations of major serum lipids and Table II the percentages of their fatty acid composition from studies in patients admitted less than 6 hours after the initial attack of pain, with mean values and statistical evaluation.

There were no significant differences between mean values on admission and on the following morning in total cholesterol, esterified cholesterol, phospholipids, or triglycerides, or in the per centages of palmitic, palmitoleic, stearic, oleic, or linoleic acids. Only one statistically significant dif ference was observed an elevation of arachidonic acid in phospholipids from 6.0 % on admission to 7.2 % on the following morning.

The results of lipid studies made less than 24 hours after onset of myocardial infarction in the two groups of patients and in the controls are tabulated in Tables III and IV

Mean total serum cholesterol was slightly higher in patients without previous CHD (group A) than in the group with old CHD (group B) and in the controls (group C), but differences were

not statistically significant (Table III) Phospholipids and triglycerides did not vary in the three groups.

Several differences were apparent in the fatty acid composition of the serum lipids (Table IV). In all three fractions, the percentage of linoleic acid was lower in group A than group B The differences were statistically highly significant and were balanced by higher percentages of some saturated and monounsaturated acids in group A, i.e., palmitic acid in triglycerides, palmitoleic acid in cholesterol esters and triglycerides, and oleic acid in cholesterol esters and phospholipids.

Quantitatively the most important differences were observed in comparisons of groups A and C. Here again, linoleic acid was lower in group A, 47 1% against 54.9% in cholesterol esters, 17 0% against 22.4% in phospholipids, and 11 8% against 14.5% in triglycerides, respectively in the two groups. These differences were also balanced by changes in the opposite direction for saturated and monounsaturated fatty acids. In group A the percentages were higher for palmitic acid in all three fractions, palmitoleic acid in cholesterol esters and triglycerides, and oleic acid in cholesterol esters.

Significant differences were also observed in some fatty acids of quantitatively minor importance. Thus, arachidonic acid was higher in phospholipids in group A than in group C.

All mean values relating to pentaenoic and hexaenoic acids were higher in group A than in group C, eicosapentaenoic in cholesterol esters, and eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in phospholipids.

DISCUSSION

In an earlier study of the fatty acid composition of adipose tissue from patients with AMI (23) it was shown that linoleic acid percentages were higher in patients with a history of previous CHD than in patients without previous cardiac disease. In the present study of similar groups from the same hospital, but from a different period, it was found that in the three major serum lipid fractions linoleic acid is also higher in patients with a history of old CHD Furthermore this group also displayed significantly lower values for palmitic, palmitoleic and oleic acids in one or more of the fractions. The results support the assumption that data concerning fatty acid composition from patients with a history of old CHD cannot be considered to reflect dietary habits prior to the onset of the disease. In this group the fatty acid composition is modified to a greater degree by recommendations given in health propaganda directed towards the whole population than in subjects who have not experienced symptoms of heart disease. Thus data from patients with an old CHD cannot be used to evaluate dietary habits of possible etiologic importance for CHD

The investigations reported here were performed less than 24 hours after the initial attack of pain. It is known that a degradation of low density lipoproteins and changes in their fatty acid composition occur a few days after the onset of myocardial infarction (9 22) but it was not considered likely that these relatively stable particles undergo significant changes in the first 24 hours. This belief is confirmed by the absence of changes in major serum lipids in patients admitted within 6 hours after the initial attack of pain. Neither the concentrations of cholesterol, phospholipids, triglycerides, nor their fatty acid composition, underwent important changes in the period from admission to the following morning. Only one statistically significant change in fatty acid composition was noted, that of arachidonic acid in phospholipids, which increased from a mean of 6.0% to a mean of 7.2%.

Thus early changes in arachidonic acid proportions following AMI may explain the difference in this fatty acid between patients in group A and controls. For the quantitatively more important fatty acids, however it is probable that the percentages found in patients with AMI as their first manifestation of disease are representative for the preinfarction fatty acid composition.

When values from this group were compared with control values, lower percentages of linoleic acid were found in all fractions, while changes in the opposite direction were seen for some saturated and monounsaturated fatty acids. The findings agree with results of an earlier study of the fatty acid composition in adipose tissue in which linoleic acid percentages were also lower in a similar group of patients than in their controls (23). However the differences in the present study appear more clearcut than in the study of adipose tissue and have high degree of statistical significance.

The findings reported here concerning fatty acid composition of serum lipids indicate particularities in the dietary habits of patients with CHD. In the Introduction to the present paper emphasis was placed on the relationship between dietary linoleate and linoleic acid in serum and the most likely explanation of the low linoleic acid percentages in the patients with myocardial infarction is a diet low in essential polyunsaturated fatty acids. However, in the dietary regulation of lipid metabolism, not only fats but carbohydrates as well are emphasized. Animal tissue cells primarily convert carbohydrates into palmitic, palmitoleic and oleic acids, but not into linoleic acid (12, 26, 31). A high sugar diet leads to decreased linoleic acid in cholesterol esters, phospholipids, and triglycerides of serum when substituted for starch (25, 32). It has thus been shown that the proportion of endogenously synthesized fatty acids increases in serum after a high sugar feed at the expense of the exogenously derived essential fatty acids. From the present study, therefore, the exact nature of the particularities characterizing the preinfarction dietary habits of the patients cannot be stated with certainty. A high sugar intake, as well as an intake of fats high in saturated and monounsaturated fatty acids and low in linoleic acid may be involved.

From the results it appears that pentaenoic and hexaenoic acids were higher in patients with AMI than in controls. The incorporation in serum lipids of dietary pentaenoic and hexaenoic acids has been less completely studied than that of linoleic acid, but in one study with fish oil fractions given to human subjects it was demonstrated that pentaenoic and hexaenoic acids compete successfully with linoleic acid and are incorporated in serum lipid fractions at the expense of this acid (14).

It has been proposed that cod liver oil and other fish oils are particularly important in the prevention of CHD (33). The present study does not support this view but indicates that the essential fatty acids may be more important in this respect than other polyunsaturated fatty acids.

In spite of the overwhelming evidence relating linoleic acid in serum and depot fat to dietary fats or carbohydrates it cannot be entirely denied that the changes in fatty acid composition are part of a constitutional metabolic disorder affecting relative incorporation in lipids. However, this objection is entirely hypothetical and lacks experimental and epidemiological support.

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FATTY ACID COMPOSITION OF SERUM LIPIDS IN WIVES OF MEN WITH MYOCARDIAL INFARCTION

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Abstract. Two groups of healthy married women, aged 40–70 years, have been studied to determine the concentrations of major serum lipid fractions and their fatty acid composition. One group consisted of wives of men with recent myocardial infarction, and the other of wives of healthy men. No significant differences were observed in cholesterol, phospholipids, or triglyceride concentrations between the two groups or in the fatty acid composition of these lipid fractions. It has previously been demonstrated that the linoleic acid percentages in the serum lipids of men with myocardial infarction are lower than in healthy men. If dietary factors are responsible for the low linoleic acid percentages in men with myocardial infarction, the reason lies in individual habits rather than in the particular dietary habits of their families.

Previous communications have demonstrated differences in fatty acid composition of serum lipids and adipose tissue between groups of patients with myocardial infarction and control subjects without known coronary heart disease (CHD) (4, 5). The most important disturbances observed in patients with myocardial infarction were low percentages of linoleic acid, and it was considered probable that the main cause of these disturbances was the patients' consumption prior to manifestation of CHD of a diet low in essential fatty acids, high in monounsaturated and saturated fatty acids and/or in simple sugars. However, the possibility of the existence of a constitutional metabolic disorder affecting the relative incorporation of fatty acids in serum and tissue lipids could not be excluded.

If diet was the main cause of the observed disturbances in fatty acid composition, it could reasonably be expected that the dietary habits leading to these changes were a reflection of the dietary conditions within the patients' families. If this was the case, presumably dietary habits and the pattern of fatty acid composition of the wives would be similar.

The present study compared the fatty acid composition of serum lipid fractions of healthy wives of patients suffering from acute myocardial infarction (AMI) as the first manifestation of CHD with that of healthy wives of healthy men.

MATERIAL AND METHODS

All 63 participants in the present study were women in the age group 40–70 years. They were healthy as evidenced by criteria described for the control material of men in a previous paper (1), and all agreed that they consumed an "ordinary diet".

One group (group A) comprised 29 wives of men who had recently experienced an AMI as the first manifestation of CHD. The remaining 34 participants were wives of healthy men (group B).

Blood for lipid analyses was drawn in the morning following a 12-hour fast, and in group A the examinations were performed within 3 days after the onset of the infarction.

The methods used in determining serum cholesterol, phospholipids, triglycerides and their fatty acid composition have been described elsewhere (2, 3). The statistical evaluation was made by *t*-tests.

Table I. *Concentrations of serum lipids in healthy women*

Mean with S.D. within parentheses

Lipid fraction (mg/100 ml)	Group A ($N = 29$)	Group B ($N = 34$)
Total cholesterol	284 (52)	268 (45)
Cholesterol esters	212 (41)	199 (37)
Phospholipids	288 (47)	272 (38)
Triglycerides	94 (47)	85 (45)

Table II. *Fatty acid composition of serum lipids in healthy women*

Mean with S.D. within parentheses

	Cholesterol esters		Phospholipids		Triglycerides	
Fatty acid (%)	Group A	Group B	Group A	Group B	Group A	Group B
Myristic					1.9 (0.7)	2.0 (0.5)
Palmitic	9.7 (1.6)	10.0 (1.0)	24.9 (2.7)	24.8 (2.1)	21.6 (2.6)	22.2 (2.6)
Palmitoleic	3.8 (1.0)	3.7 (0.9)	1.5 (0.5)	1.4 (0.5)	5.9 (1.1)	5.7 (1.3)
Stearic	1.2 (0.3)	1.3 (0.5)	14.0 (1.4)	14.6 (2.0)	5.7 (0.7)	5.7 (1.0)
Oleic	20.1 (2.4)	19.9 (3.4)	12.1 (1.4)	[illegible] (1.8)	40.3 (2.4)	39.9 (3.9)
Linoleic	52.3 (4.6)	53.9 (6.3)	20.3 (2.6)	21.4 (3.7)	14.2 (3.7)	14.9 (4.4)
Eicosenoic					1.3 (0.5)	1.2 (0.6)
Eicosatrienoic			2.4 (0.6)	2.6 (0.9)		
Arachidonic	5.5 (1.3)	5.1 (1.2)	6.5 (1.4)	6.8 (1.4)	1.0 (0.4)	0.9 (0.3)
Behenic			1.8 (1.2)	1.6 (1.1)		
Eicosapentaenoic	2.1 (1.2)	1.6 (0.9)	2.9 (0.8)	2.7 (0.9)		
Docosapentaenoic			2.8 (1.2)	2.3 (1.1)		
Docosahexaenoic			4.6 (1.3)	4.7 (1.7)		

RESULTS AND DISCUSSION

The results of the determination of cholesterol, phospholipids, triglycerides, and of the fatty acid composition of these fractions, are tabulated in Tables I and II. No statistically significant differences between means were observed between the two groups.

The study therefore gives no indication that wives of men with myocardial infarction have dietary habits differing from those of wives of healthy men.

Thus, if dietary factors are responsible for the low linoleic acid percentages previously demonstrated in men with myocardial infarction, it is based on individual habits rather than on the particular dietary habits of their families. However the negative result of the present study does not contribute to the problem concerning the cause of low linoleic acid values. If similar findings had also been made in the wives, the evidence for dietary origin would have been good.

Since the fatty acid composition in group A was the same as in group B the question of whether the disturbances in fatty acid composition in the men with myocardial infarction are caused by dietary factors or by some unknown constitutional disorder remains obscure.

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DISTURBANCES IN SERUM LIPIDS AND IN THEIR FATTY ACID COMPOSITION FOLLOWING ACUTE MYOCARDIAL INFARCTION

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Abstract. Disturbances in serum lipids and in their fatty acid composition have been studied during the early phase of acute myocardial infarction. A rise in free fatty acids was observed within 2–6 hours after onset of pain, and on the 3rd–4th day a decline in concentrations of cholesterol and phospholipids together with changes in their fatty acid composition. Linoleate percentages decreased, while arachidonate and palmitate increased. For triglycerides elevations in palmitate and oleate and a decline in linoleate were observed in the same period. It is concluded that an increase in β-lipoprotein degradation—and not a decline in their synthesis—is the mechanism responsible for lowering of cholesterol and phospholipids. Various endocrine factors occurring in response to stress may be involved in the mechanisms leading to lipoprotein degradation, but thyroxine is the only hormone having nearly the same effect on lipid metabolism as that of acute serious illness. It is postulated that alterations in the free thyroxine level contribute more to the homeostatic response to serious illness than has been generally believed hitherto, and that thyroxine is the main cause of the changes in β-lipoproteins observed after myocardial infarction.

In serious illness or trauma, marked changes have been observed in free fatty acids (FFA), β-lipoproteins and pre-β-lipoproteins in serum.

Acute myocardial infarction (AMI) causes increased concentrations of FFA, which reach a maximum level a few hours after the onset of pain (23); a rise in FFA has also been reported following burns (2) and surgery (36).

β-lipoproteins, cholesterol and phospholipids tend to decrease a few days after the onset of myocardial infarction (3, 9, 33, 39) and similar findings have been made following surgery (26), burns (2) and during acute exacerbation of chronic respiratory failure (28).

The disturbances observed in fasting triglyceride concentrations after a myocardial infarction have not been as clearcut as those of cholesterol and phospholipids, but a rise in triglyceride-rich pre-β-lipoproteins and a delayed rise in triglycerides have been observed (9, 33). Birke et al. (2) found no statistically significant changes in triglycerides following burns.

The mechanisms and reasons for the decrease in β-lipoproteins, cholesterol and phospholipids following serious illness are obscure. A decline in synthesis, or an acceleration of degradation of the β-lipoproteins or their components, may be involved. In burns Birke et al. (2) found that the decrease in cholesterol was most pronounced at the time when signs of liver damage were present, and suggested that a decreased hepatic synthesis of lipoproteins was responsible for the low levels of cholesterol and phospholipids.

In a previous study (22) it has been shown that changes in fatty acid composition of the serum lipids occur at an early stage of myocardial infarction, with a fall in linoleic acid percentages. The change in fatty acid composition indicates an active resynthesis of lipoproteins. However, in evaluating these disturbances the time factor of the various changes may be important. In the present study, therefore, serial determinations of serum lipids and their fatty acid composition have been performed in the first 4 days following onset of myocardial infarction.

MATERIAL AND METHODS

Twelve men, aged 40 to 70 years, with AMI were studied. Only subjects admitted to hospital less than 6 hours (average 4 hours) *after the initial attack of pain* were included. Diagnosis was based on standard criteria, with the characteristic pain, ECG changes, S-GOT

Table I. *Serum lipids after myocardial infarction (mg/100 for FFA µEq/l)*

Mean with S.D. for values on admission within parentheses, ns = not significant

	Not fasting				Fasting			Statistical evaluation		
	A. Admission	B. 8 h	C. 19 h	D 26 h	E. Day 2	F Day 3	G Day 4	AE	AF	AG
Total cholesterol	302 (44)	300	298	289	298	281	240	ns	ns	$p < 0.05$
Cholesterol esters	205 (35)	203	202	196	203	187	160	ns	ns	$p < 0.05$
Phospholipids	273 (41)	269	268	261	260	239	214	ns	$p < 0.05$	$p < 0.01$
Triglycerides	96 (39)	105	99	102	94	89	92	ns	ns	ns
FFA	1 600 (512)	880	1 053	823	904	809	714	$p < 0.05$	$p < 0.01$	$p < 0.01$

elevation to 50 units or more, and elevation of ESR, WBC and temperature. Patients known to have diabetes mellitus, hypothyroidism, or renal disease, as well as patients showing glucosuria upon admission, were excluded from the study. So were also patients complaining of nausea and lack of appetite, and all participants admitted to having normal appetite. They were placed on an ordinary (not cholesterol-lowering) hospital diet.

Blood for lipid analysis was drawn on admission and 8, 19 and 26 hours later. These blood samples (A to D in the Tables) were not obtained in the fasting condition, but samples following a 12-hour fast were drawn in the morning of days 2, 3 and 4 (E to G in the Tables).

FFA were determined according to the method of Dole (10) as modified by Trout et al. (34). The procedure for triglyceride determination was a modification of the method described by Carlson and Wadström (7). The method adopted for the determination of total and esterified cholesterol was that of Webster (38), phospholipids were determined by the method of Brun (5). The procedure used for determining fatty acid composition of serum lipids by means of gas chromatography has been described earlier (21).

The statistical evaluations were performed using *t*-tests for paired data.

RESULTS

Table I tabulates mean values for concentrations of FFA, total cholesterol, esterified cholesterol phospholipids, and triglycerides. Standard deviations are tabulated only for the percentages obtained on admission.

Peak FFA values were found in blood samples drawn less than 6 hours after onset of pain. They were very high, with a mean of 1 600 µEq/l, and considerably higher than those observed in the fasting condition on day 2. FFA values were also abnormally high in most patients on the second and third days, but on the fourth day most values were within the normal range.

Total cholesterol and cholesterol esters showed a lowering of values, with differences between means between the initial value and that obtained on the 4th day which are statistically significant. Phospholipids also declined, and the differences between means were statistically significant when the initial value was compared with values on days 3 and 4. No significant differences between means of triglyceride concentrations were observed.

The fatty acid composition of cholesterol esters, phospholipids and triglycerides are tabulated in Tables II, III and IV. Several changes occurred in the period studied. In cholesterol

Table II. *Fatty acid composition of cholesterol esters after myocardial infarction (%)*

Mean with S.D. of values on admission within parentheses, ns = not significant

	Not fasting				Fasting			Statistical evaluation		
	A. Admission	B. 8 h	C. 19 h	D 26 h	E. Day 2	F Day 3	G. Day 4	AE	AF	AG
Palmitic	9.9 (1.9)	9.6	9.6	10.1	9.8	10.5	10.7	ns	ns	p 0.05
Palmitoleic	3.6 (1.3)	3.6	3.6	3.7	3.6	3.6	3.5	ns	ns	ns
Stearic	0.8 (0.5)	0.8	0.8	0.8	0.8	0.8	0.9	ns	ns	ns
Oleic	20.2 (3.4)	19.9	19.7	20.2	19.8	19.9	20.5	ns	ns	ns
Linoleic	53.4 (6.3)	54.1	54.1	53.1	54.0	52.4	51.3	ns	ns	$p < 0.05$
Arachidonic	5.3 (1.4)	5.6	5.6	5.7	5.6	5.7	6.0	ns	ns	$p < 0.05$
Eicosapentaenoic	3.4 (1.9)	3.4	3.6	3.6	3.6	3.5	3.5	ns	ns	ns

Table III. *Fatty acid composition of phospholipids after myocardial infarction* (%)

Mean with S.D. of values on admission within parentheses; ns = not significant

	Not fasting				Fasting			Statistical evaluation		
	A. Admission	B. 8 h	C. 19 h	D. 26 h	E. Day 2	F. Day 3	G. Day 4	AE	AF	AG
Palmitic	25.8 (2.5)	25.8	25.9	27.1	26.1	27.3	27.7	ns	ns	$p<0.01$
Palmitoleic	1.4 (0.4)	1.2	1.0	1.2	1.3	1.2	1.4	ns	ns	ns
Stearic	12.0 (1.2)	14.0	13.3	12.6	11.8	11.6	11.9	ns	ns	ns
Oleic	12.0 (1.5)	12.1	11.1	11.6	11.8	11.9	11.9	ns	ns	ns
Linoleic	20.9 (3.4)	20.6	20.0	19.8	19.8	19.3	17.5	ns	ns	$p<0.05$
Eicosatrienoic	2.4 (0.7)	2.4	2.4	2.3	2.4	2.3	2.4	ns	ns	ns
Arachidonic	6.0 (1.1)	6.8	7.0	6.8	7.3	6.9	6.4	$p<0.001$	ns	ns
Eicosapentaenoic	3.7 (1.4)	3.3	3.9	3.7	3.7	3.5	3.4	ns	ns	ns
Docosapentaenoic	2.7 (0.9)	2.3	2.5	2.6	2.4	2.4	2.8	ns	ns	ns
Docosahexaenoic	6.1 (2.3)	6.0	6.7	6.6	6.7	6.8	6.7	ns	ns	ns

esters and phospholipids linoleic acid decreased and palmitic acid increased, with differences between means which are statistically significant when values obtained initially and on the 4th day were compared. For triglycerides the increase in palmitic and oleic acid and the decrease in linoleic acid are statistically significant.

In cholesterol esters and phospholipids elevations were observed in percentages of arachidonic acid. In the phospholipids these changes took place early with a significant increase from admission to the second day. The highest values for cholesterol esters were found on day 4.

It is evident from the Tables that, with the exception of FFA concentrations and phospholipid arachidonate percentages, no important changes in concentrations of lipid fractions or fatty acid composition of these lipids occur in the first 26 hours following onset of myocardial infarction.

DISCUSSION

The study demonstrates the disturbances in serum lipids after myocardial infarction. Maximum FFA values were observed as early as 2 to 6 hours after onset of pain. No other disturbances in the concentrations of major lipids were observed in the first 26 hours. In a later phase—on the 3rd and 4th day—values of cholesterol and phospholipids decreased, phospholipids possibly declining more rapidly than cholesterol. The fatty acid composition of these lipids displayed important changes on the 3rd and 4th day with a lowering of linoleic acid and an increase in the palmitic acid percentage. No significant change was observed in triglyceride concentrations in the 4 days of the study but fatty acid composition was also altered in this fraction, with an increase in palmitic and oleic acid and a decrease in linoleic acid.

The endocrine disturbances known to be involved in somatic stress offer satisfactory explanations for the high FFA values. The origin of these FFA is mainly lipolysis of adipose tissue, and the degree of lipolysis depends upon lipases activated by adenosine 3',5'-monophosphate (cyclic AMP). According to Sutherland et al. (30) the formation of cyclic AMP is stimulated by catecholamines, ACTH, TSH and thyroxine. Insulin induces a lowering of cyclic AMP and inhibits lipolysis. Growth hormone potentiates the lipolytic effect of other hormones (11).

It is well known that stress has a stimulating effect upon the excretion of catecholamines and ACTH and in the early phase of myocardial infarction increased concentrations of catecholamines and corticoids have been demonstrated in plasma as well as in urine (25 27 35). Growth hormone concentrations have also been shown to increase as a direct effect of the stress of myocardial necrosis, whether or not glucose utilization is depressed (24). Patients in shock have a reduced secretion of insulin (31).

The thyroid function has not been adequately studied under conditions of stress. The turnover of thyroxine is slower than that of ACTH, catecholamines, insulin, and growth hormone, and it has been generally believed that the thyroid gland does not respond as rapidly as the adrenal

Table IV *Fatty acid composition of triglycerides after myocardial infarction* (%)

Mean with S.D. of values on admission within parentheses. ns = not significant

	Not fasting				Fasting			Statistical evaluation		
	A. Admission	B. 8 h	C. 19 h	D. 26 h	E. Day 2	F. Day 3	G. Day 4	AE	AF	AG
Palmitic	20.5 (2.5)	20.9	22.3	22.4	22.0	22.6	22.4	$p<0.05$	$p<0.05$	$p<0.05$
Palmitoleic	4.9 (1.0)	5.4	5.1	5.5	5.3	4.9	4.6	ns	ns	ns
Stearic	5.8 (1.0)	5.0	4.8	5.1	5.1	4.8	4.4	ns	ns	ns
Oleic	40.6 (3.5)	40.6	41.7	41.0	41.3	42.2	43.7	ns	ns	$p<0.05$
Linoleic	17.4 (3.5)	17.4	16.7	16.3	17.1	15.8	15.1	ns	ns	$p<0.05$
Linolenic	1.4 (0.4)	1.3	1.1	1.1	1.0	0.8	0.7	ns	ns	ns
Docosahexaenoic	2.0 (1.2)	2.6	2.0	2.0	2.1	2.0	2.3	ns	ns	ns

medulla and cortex to stress with augmented secretion. However it has been shown that the turnover of thyroxine and triiodothyronine is accelerated during febrile illness—a finding suggesting a consequent increase in the secretion of TSH and thyroidal hormones (12). A pattern of disturbances in thyroid function in seriously ill patients has recently been described, showing a decrease in the capacity of thyroxine-binding globulin and raised free thyroxine levels (in the "thyrotoxic" range) but with small changes in total thyroxine levels (13). It is possible, there re, that alterations, not only in turnover of al circulating thyroxine but also in the binding f thyroxine by proteins, provide mechanisms for rapid modulations of the concentrations of free thyroxine in response to stress. The suggestion has been made but not confirmed, that FFA, in competing with binding sites on serum proteins, influence the capacity of thyroxine binding (4, 14).

It is evident that many of the endocrine disturbances observed in severe illness may be involved in the activated lipolysis leading to high FFA concentrations in plasma after myocardial infarction. Catecholamines are probably more important than other factors in this respect since sympathetic inhibitors inhibit the posttraumatic rise in FFA (6).

The rise in triglyceride-rich pre-β-lipoproteins following myocardial infarction (9) may be caused by the large amounts of FFA available for formation of triglycerides in the liver. In the present study the finding that oleic acid percentages in triglycerides increase at an early stage agrees with the concept that FFA originating from a stimulated lipolysis of oleic acid rich adipose tissue are related to the rise in triglyceride formation.

However in the first week the changes in fasting triglyceride concentrations appeared small and insignificant (33) and it is in a later phase that values rise. In fact, in the early phase triglyceride concentrations may be influenced by opposing factors—on the one hand the increase in pre-β-lipoproteins, on the other the lowering of β-lipoproteins which also carry triglycerides.

The factors responsible for the lowering of β-lipoproteins, cholesterol and phospholipids are obscure. In a previous communication the question of whether or not dietary factors were of importance was unresolved (22). Patients with myocardial infarction sometimes complain of nausea and lack of appetite, which may affect intake of food, including fats. However the short-time effect of a low-calory low-fat diet on serum cholesterol is very small and inconsistent (37), and it is highly unlikely that the marked lowering of cholesterol and phospholipids following infarction is caused by diet. Patients with lack of appetite and nausea were excluded from the present study. The participants claimed that they ate as much as usual and they were placed on an ordinary diet. In these patients, therefore the possibility of diet being responsible for the large fall in serum lipids in the first 3–4 days after onset of infarction can be excluded.

From the results of the present study it is apparent that disturbances in fatty acid composition in cholesterol esters and phospholipids occur as early as those of the concentrations of these lipids. The changes in fatty acid composition indicate an active synthesis of lipoproteins, and a decrease in hepatic synthesis as the main cause

of the decline in β-lipoproteins, cholesterol and phospholipids seems highly improbable.

The factor(s) responsible for these changes, therefore, probably act upon lipoprotein degradation. Many of the hormones involved in the homeostatic mechanisms of stress are known to affect lipid metabolism, and it is appropriate to discuss endocrine factors also in the pathogenesis of the disturbances of cholesterol and phospholipids described here.

It has been reported that ACTH produces a small drop in total serum cholesterol without causing important changes in phospholipids (1 8 29). Very little is known of the effect of this hormone on fatty acid composition but experiments in rabbits showed that large doses of ACTH over a 5-day period failed to induce changes similar to those observed following myocardial infarction (20).

After noradrenaline infusion some changes in serum lipids were observed, with a decrease in phospholipid concentrations and phospholipid palmitate, and increases in the percentages of triglyceride oleate and cholesterol and phospholipid arachidonate (16). Serum cholesterol did not react significantly to an epinephrine load (32)

Human growth hormone has been reported as effecting changes in serum lipids, i.e. decreases in phospholipid concentrations, and in the levels of phospholipid olate, but increases in triglyceride palmitate cholesterol oleate, phospholipid lineolate and eicosatrienoate (15).

Thus ACTH catecholamines and growth hormone may induce changes in serum lipids similar in some respects to those observed following myocardial infarction, but differing in other parameters.

However the changes induced by thyroid hormones are in close agreement with those following myocardial infarction. Lowering of cholesterol and phospholipids, decrease in linoleic acid, and increase in saturated and monounsaturated fatty acids, as well as in arachidonic acid, are seen in thyrotoxicosis, following administration of thyroxine, and in AMI (17 18).

It is probable that the effect of serious illness on serum lipoproteins is a complex and combined effect of various endocrine factors, but since the thyroid hormones have nearly similar effects on lipid metabolism, and free thyroxine levels have been reported within the "thyrotoxic range following serious illness (13) this hormone may well be the key factor in this connection.

The effect of thyroxine offers an explanation for the lowering of linoleate percentages following myocardial infarction. Thyroxine accelerates lipoprotein degradation and fatty acid oxidation (including linoleate) and induces increases in lipoprotein and fatty acid synthesis. The latter phenomenon would fa our the incorporation in lipoproteins of fatty acids synthesized in the body mainly saturated and monounsaturated. In addition, the activated lipolysis increases the availability of palmitic and oleic acids. Furthermore, the stimulation of arachidonate synthesis from linoleate by thyroxine may contribute to the drop in linoleic acid.

Disturbances in lipoprotein degradation following acute serious illness have also been shown to occur in patients suffering from hypothyroidism (19). This is not necessarily a contradiction of the hypothesis that thyroxine is the major factor in causing lipoprotein degradation under these conditions Changes in binding capacity of the thyroxine-binding proteins may produce alterations in free thyroxine levels in hypothyroid as well as in euthyroid subjects.

Direct measurements of thyroid parameters—including free thyroxine and capacity of thyroxine–binding proteins in patients with myocardial infarction—may confirm the hypothesis.

If the contribution of thyroxine to the homeostatic response to serious illness is greater than hitherto believed, the consequences for patients with myocardial infarction may be considerable, since free thyroxine levels in the "thyrotoxic" range would also affect oxygen consumption.

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CONCENTRATIONS AND FATTY ACID COMPOSITION OF SERUM LIPID FRACTIONS FOLLOWING ACTH IN RABBITS

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Abstract. In a group of rabbits injection of ACTH over 5 days caused a rise in cholesterol, phospholipids and triglycerides (TG) in serum. Concomitant changes in fatty acid composition included an increase in linoleic acid in cholesterol esters and phospholipids, a lowering of oleic acid in all three fractions, and an increase in palmitic acid in TG. The changes in fatty acid composition may be accounted for by increased lipolysis of adipose tissue effected by ACTH with an increased influx to the liver of free fatty acids (FFA), rich in palmitic and linoleic acid, and relatively poor in oleic acid. Furthermore, inhibition by ACTH of fatty acid synthesis may have favoured the incorporation of linoleic acid at the expense of fatty acids synthetized in the liver. It is concluded that the changes in lipid concentrations and fatty acid composition after injecting ACTH in rabbits are quite different from those observed following serious illness in man. This may indicate that the degradation of low density lipoproteins, with lowering of cholesterol and phospholipids following serious illness in man, is not caused by ACTH or corticosteroids.

Serious illness and trauma effect marked changes in serum lipids. A lowering of cholesterol, phospholipids and low density lipoproteins (LDL) occurs in the first week following myocardial infarction, burns or surgery (4, 5, 10, 14, 18, 20).

The triglyceride (TG)-rich, very low density lipoproteins (VLDL) apparently undergo inverse changes in myocardial infarction, with an increase in concentrations (10).

A concomitant change in fatty acid composition of these lipids, with an initial decline in the percentages of linoleic acid, has also been observed in myocardial infarction (13).

The rise in VLDL is probably related to a hormone-induced increase in lipolysis, which releases free fatty acids (FFA) for TG formation in the liver.

The change in fatty acid composition indicates that the fall in LDL concentrations is caused by an enhancement of their degradation, but the mechanisms responsible for this degradation are obscure. They may be related to one or more of the endocrine reactions to stress which are known to include enhanced production of ACTH.

In an attempt to elucidate one of the problems concerning the changes in lipoproteins following somatic stress, the concentrations and fatty acid composition of serum lipid fractions have been studied following the administration of ACTH in rabbits.

MATERIAL AND METHODS

A group of eight healthy rabbits of both sexes, weighing 3.5–4 kg at the start of the experiment, were given 1 mg Synacthen Depot intramuscularly on 5 successive mornings. On the morning on which the experiment began and on the 6th, 11th and 22nd days, blood was drawn for lipid studies. The animals were weighed on the same days.

Another group of 8 rabbits served as controls and underwent the same procedures, with the exception that the injections of ACTH were replaced by a puncture with a needle on an empty syringe.

The animals received food and water ad lib. They were fed a commercial chow containing 3.5% crude fat, with a fatty acid composition of myristic acid 0.9%, palmitic 15.5%, palmitoleic [illegible].6%, stearic 1.8%, oleic 19.1%, linoleic 37.3%, linolenic 20.[illegible]% and eicosenoic acid 2.6%.

Total and esterified cholesterol was determined according to the method of Webster (19), and phospholipids by the method of Bran (7). The procedure adapted for the determination of TG was a modification of the method of Carlson and Wadström (8).

The procedure for separation and gas-chromatography in determining fatty acid composition of lipid fractions has been described previously (12).

Table I. *Cholesterol, phospholipids and triglycerides (mg/100 ml) and weight (g) in ACTH-treated rabbits and controls*

Means with S.E.M. within parentheses

Day	Total cholesterol	Esterified cholesterol	Phospholipids	TG	Weight
ACTH-treated					
1	63 (9)	55 (6)	125 (12)	59 (15)	3 765
6	112 (15)	72 (8)	181 (23)	292 (99)	3 673
11	84 (12)	58 (7)	156 (18)	112 (36)	3 629
22	83 (8)	60 (6)	128 (7)	51 (12)	3 909
Controls					
1	55 (8)	42 (7)	123 (10)	60 (15)	3 581
6	64 (8)	50 (6)	117 (7)	61 (17)	3 650
11	58 (8)	46 (6)	115 (10)	52 (14)	3 735
22	69 (10)	52 (9)	123 (11)	49 (12)	3 938

RESULTS

Table I shows the mean concentrations of total and esterified cholesterol, phospholipids and TG in the animals given ACTH and in the control group. The Table also includes the mean weights of the animals.

It appears that ACTH effected an increase in concentrations of all lipid fractions, with a change in mean values from the 1st to the 6th day from 63 to 112 mg/100 ml in total cholesterol, from 125 to 181 mg 100 ml in phospholipids, and from 59 to 292 mg/100 ml in TG There were large individual variations in this response particularly in TG as evidenced by the large standard error of mean observed on the 6th day

A loss of weight in the rabbits receiving ACTH contrasted with the gradual increase in weight in the control animals.

Tables II, III and IV show the fatty acid composition of cholesterol esters, phospholipids and TG respectively Administration of ACTH had significant effects in all fractions. In cho-

Table II. *Fatty acid composition of cholesterol esters (%) in ACTH-treated rabbits and controls*

Means with S E.M within parentheses

	ACTH-treated				Controls			
Day	1	6	11	22	1	6	11	22
Palmitic	14.5 (0 4)	15.3 (0.5)	15 1 (0.5)	16.0 (0.5)	14 1 (0.4)	14.6 (0 6)	15.3 (0 4)	15.9 (0.5)
Palmitoleic	3.3 (0.4)	3.2 (0.3)	2.9 (0.3)	2.7 (0.3)	2.8 (0.4)	2.1 (0.4)	2.5 (0.3)	3.0 (0 3)
Stearic	2.4 (0.1)	2 8 (0.2)	2.4 (0.2)	2.8 (0.2)	2.4 (0.1)	3.0 (0.1)	2.5 (0 2)	3.0 (0.1)
Oleic	17.6 (1.6)	13.8 (0 8)	17.0 (1 1)	16.9 (1.0)	18.9 (1.3)	18 3 (1.4)	18 8 (1 2)	18.1 (0 9)
Linoleic	40.2 (1 6)	46.2 (1 6)	42 5 (1 7)	43 4 (1.5)	40.0 (1.5)	41.0 (1 4)	39 9 (1.6)	40.5 (1.5)
Linolenic	8.5 (1.0)	9.0 (1.0)	8 7 (0.9)	9.2 (0.8)	7.8 (1 1)	7 6 (0.9)	7.8 (0.9)	7 4 (1.0)
Arachidonic	1 9 (0.1)	1 9 (0 1)	2.2 (0 1)	2.1 (0.1)	1.6 (0.1)	2.2 (0.1)	2 1 (0.1)	2 1 (0.1)
Docosenoic?	2.1 (0.1)	1.6 (0.2)	1.6 (0.1)	1.8 (0.1)	1 7 (0.1)	1.5 (0.1)	1.5 (0.1)	1 6 (0.2)

Table III. *Fatty acid composition of phospholipids (%) in ACTH-treated rabbits and controls*
Means with S.E.M. within parentheses

	ACTH-treated				Controls			
	1	6	11	22	1	6	11	22
Palmitic	23.3 (0.7)	25.4 (0.9)	24.5 (0.8)	24.8 (0.7)	23.0 (1.0)	22.7 (0.8)	22.3 (0.8)	23.5 (0.7)
Stearic	21.4 (1.2)	23.7 (0.7)	22.7 (0.9)	21.7 (0.9)	21.0 (0.8)	21.4 (0.8)	21.8 (0.9)	20.6 (0.7)
Oleic	11.8 (0.9)	7.2 (0.9)	9.5 (1.0)	10.7 (1.1)	10.6 (0.8)	10.6 (0.9)	10.6 (1.0)	11.1 (0.9)
Linoleic	25.0 (0.8)	29.7 (0.8)	25.9 (0.9)	4.8 (0.9)	28.9 (1.1)	29.1 (1.0)	27.8 (1.1)	28.2 (0.9)
Linolenic	2.4 (0.3)	2.4 (0.2)	2.4 (0.2)	2.3 (0.3)	2.7 (0.2)	2.6 (0.3)	2.7 (0.3)	[illegible] (0.3)
Arachidonic	3.5 (0.4)	2.9 (0.5)	3.7 (0.4)	3.3 (0.5)	3.7 (0.3)	3.9 (0.4)	1.7 (0.3)	3.2 (0.4)
Docosenoic ?	2.0 (0.2)	1.2 (0.2)	1.6 (0.3)	1.8 (0.2)	1.9 (0.2)	1.8 (0.3)	1.9 (0.3)	2.0 (0.2)
Docosahexaenoic	2.7 (0.3)	2.4 (0.3)	3.0 (0.4)	2.2 (0.3)	2.5 (0.3)	2.0 (0.2)	2.3 (0.3)	1.9 (0.3)

lesterol esters linoleic acid increased from 40.2 to 46.2% ($p<0.05$) and oleic acid decreased from 17.6 to 13.8% ($p<0.05$). Similar changes were observed in phospholipids, with an increase in linoleic acid from 25.0 to 29.7% ($p<0.05$) and a decrease in oleic acid from 11.8 to 7.2% ($p<0.05$). *In TG the administration of ACTH* also resulted in a decrease in oleic acid, from 26.2 to 21.5% ($p<0.05$) and an increase in linoleic acid, from 19.7 to 23.3%, the difference however being statistically insignificant. One additional change occurred in TG, that of palmitic acid, which rose from 26.0 to 32.2% ($p<0.01$).

After termination of the ACTH treatment the values of lipid concentrations and fatty acid composition showed a tendency to return to their original patterns.

In the control animals no change occurred either in concentrations or in fatty acid composition during the observation period.

Table IV also shows mean percentages from the determination of fatty acid composition of adipose tissue in the control animals.

DISCUSSION

There are many obscure aspects concerning the effect of ACTH and corticosteroids on lipid metabolism. One effect of these hormones is to stimulate lipolysis in adipose tissue. The subsequent increase in FFA in serum and the greater influx of FFA to the liver offer an explanation of the hypertriglyceridemia observed in the animals given ACTH in the present study and the hyperlipemia observed in experiments performed with cortisone in rabbits (15). In the latter experiment the rise in lipid concentrations began very early and was usually apparent by the third day.

This explanation would be similar to that given for the rise in TG-rich VLDL following myocardial infarction in man. In this condition, too, hormonal factors effect an increase in lipolysis and FFA concentrations, which subsequently become available for TG formation in the liver.

A lipolysis of adipose tissue would also offer an explanation of the changes in fatty acid composition of serum TG in the rabbits. The statistically significant changes following ACTH were an increase in palmitic acid and a decrease in oleic acid. *In adipose tissue from the rabbits* (Table IV) the percentages of palmitic acid were high and those of oleic acid low compared with the values in serum TG. The changes occurring in serum TG fatty acid composition after ACTH, therefore, are those one would expect if the

Table IV *Fatty acid composition (%) of serum triglycerides in ACTH-treated rabbits and controls, and of adipose tissue in controls*

Means with S.E.M. within parentheses

	ACTH-treated				Controls				
Day	1	6	11	22	1	6	11	22	Adipose tissue
Myristic	1.4 (0.2)	1.3 (0.1)	1.4 (0.1)	1.8 (0.1)	1.3 (0.1)	1.3 (0.1)	1.4 (0.1)	1.5 (0.1)	2.5 (0.1)
Palmitic	26.0 (1.2)	32.3 (1.3)	31.4 (1.4)	30.1 (1.3)	27.1 (1.2)	26.9 (1.3)	28.2 (1.2)	27.8 (1.1)	31.9 (1.3)
Palmitoleic	3.4 (0.5)	3.0 (0.3)	2.9 (0.4)	2.9 (0.3)	3.2 (0.4)	3.0 (0.3)	3.1 (0.5)	2.9 (0.5)	4.7 (0.6)
Stearic	4.9 (0.4)	5.7 (0.6)	6.1 (0.6)	5.9 (0.5)	5.1 (0.4)	5.4 (0.5)	4.9 (0.4)	5.3 (0.5)	7.0 (0.4)
Oleic	26.2 (1.5)	21.5 (0.9)	22.6 (1.0)	23.7 (1.2)	25.8 (1.4)	26.3 (1.5)	25.1 (1.5)	26.9 (1.5)	20.4 (1.0)
Linoleic	19.7 (1.6)	23.3 (1.2)	21.3 (1.3)	18.3 (1.3)	19.3 (1.5)	18.7 (1.3)	19.6 (1.2)	19.2 (1.3)	19.8 (0.6)
Linolenic	9.0 (0.5)	8.4 (0.3)	7.4 (0.3)	6.9 (0.3)	8.5 (0.5)	8.7 (0.4)	7.8 (0.4)	8.1 (0.3)	11.2 (0.6)
Eicosenic	1.1 (0.2)	0.8 (0.2)	0.9 (0.1)	1.1 (0.2)	1.0 (0.1)	0.8 (0.1)	1.1 (0.2)	0.9 (0.1)	1.3 (0.1)

proportions of incorporated fatty acids from adipose tissue increased. In explaining the lowering of oleic acid values, the possibility of an inhibition of synthesis of this acid by ACTH must also be considered. It has been demonstrated that cortisol inhibits fatty acid synthesis in the liver (3 6) and that the incorporation of glucose ... into fatty acids is inhibited by dexamethasone (11). An inhibition of fatty acid synthesis following ACTH may have affected the amount of oleic acid available for TG formation.

The rise in cholesterol and phospholipids following ACTH was smaller than that of TG but was statistically significant. The finding of hypercholesterolemia following ACTH is in accordance with other studies in rabbits, demonstrating a rise in cholesterol levels effected by cortisone (15)

Significant changes in fatty acid composition occurred in cholesterol esters and phospholipids during the administration of ACTH in both fractions with a rise in linoleic acid and a decrease in oleic acid percentages. Linoleic acid is an essential fatty acid originally derived from the diet. In the present study the rabbits given ACTH lost weight and showed a negative calory balance, and it is therefore unlikely that the rise in linoleic acid percentages was caused by an increase in the proportion between dietary intake and degradation of this acid. The rise probably originates from the lipolysis of the relatively linoleate-rich adipose tissue. An inhibition of fatty acid synthesis would favour the incorporation of linoleic acid at the expense of oleic and other fatty acids partly synthesized in the liver

From the results of the present study in rabbits it may be concluded that the effects of ACTH on serum lipid concentrations and fatty acid composition (hypercholesterolemia, hyperphospholipidemia, increase in linoleic acid) are far different from those observed following serious illness in man (decreased cholesterol, phospholipids and linoleic acid). The present results may indicate that ACTH or corticosteroids are not the endocrine factors responsible for the degradation of LDL in somatic stress.

However this conclusion is by no means positive Differences between species have been reported in the reaction of lipid metabolism to ACTH and cortisone. In man these hormones have been reported to effect decreases as well as increases in serum cholesterol (1 9 16 17). Many reports ha e been based on experiments in seriously ill patients, and an observed lowering of serum lipids may ha e been due to somatic

stress rather than to the administered hormones. A thorough study of the effect of these hormones on lipid concentrations and fatty acid composition in healthy human subjects has yet to be undertaken.

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Means with S.E.M within parentheses

	ACTH-treated				Controls				
Day	1	6	11	22	1	6	11	22	Adipose tissue
Myristic	1.4 (0.2)	1.3 (0.1)	1.4 (0.1)	1.8 (0.1)	1.3 (0.1)	1.3 (0.1)	1.4 (0.1)	1.5 (0.1)	2.5 (0.1)
Palmitic	26.0 (1.2)	32.3 (1.3)	31.4 (1.4)	30.1 (1.3)	27.1 (1.2)	26.9 (1.3)	28.2 (1.2)	27.8 (1.1)	31.9 (1.3)
Palmitoleic	3.4 (0.5)	3.0 (0.3)	2.9 (0.4)	2.9 (0.3)	3.2 (0.4)	3.0 (0.3)	3.1 (0.5)	2.9 (0.3)	4.7 (0.6)
Stearic	4.9 (0.4)	5.7 (0.6)	6.1 (0.6)	5.9 (0.5)	5.1 (0.4)	5.4 (0.5)	4.9 (0.4)	5.3 (0.5)	7.0 (0.4)
Oleic	26.2 (1.5)	21.5 (0.9)	22.6 (1.0)	23.7 (1.2)	25.8 (1.4)	26.3 (1.5)	25.1 (1.5)	26.9 (1.5)	20.4 (1.0)
Linoleic	19.7 (1.6)	23.3 (1.2)	21.3 (1.3)	18.3 (1.3)	19.3 (1.5)	18.7 (1.3)	19.6 (1.2)	19.2 (1.3)	19.8 (0.6)
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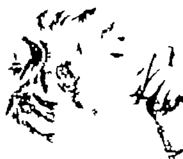
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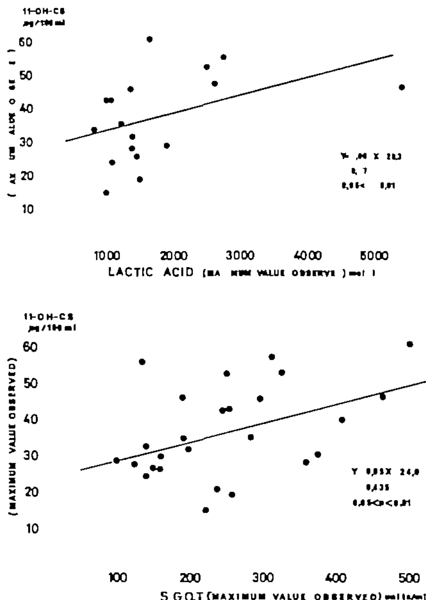


Fig. 2. The individual peak plasma 11-OH-CS concentrations in AMI patients plotted against their peak venous blood lactate values (*a*) and against peak SGOT values (*b*). The equation of the regression line, the correlation coefficient and the *p*-value of the correlation are shown.

A relationship was found between the peak values observed for plasma cortisol and peak SGOT levels or peak blood lactate concentrations (Fig. 2).

DISCUSSION

The cortisol secretion rate is the most accurate measurement of the adrenal cortical activity available to-day. Measurement in plasma does, however, allow definition of rapid changes in the activity of the glandular apparatus. This study shows that in uncomplicated myocardial infarction there is a slight and transitorily increased cortisol secretion if it is assumed that the increased plasma levels reflect increased production and not decreased metabolism. In more seriously affected patients plasma cortisol levels are more distinctly elevated and may rise to 2–3 times

above normal. Our findings amplify and extend the earlier reports by Klein and Palmer (5) and Logan and Murdoch (6). It is notable, however that the highest plasma cortisol values in our patients were lower than those reported by these authors. They found that in the presence of cardiogenic shock the adrenals may produce mean plasma cortisol levels 3–17 times above normal. In uncomplicated myocardial infarction plasma cortisol was increased to 1–3 times above normal values. The discrepancy between the elevation of plasma cortisol in our patients and in other studies in this field may reflect differences in the patients selected for the study.

The elevation of plasma cortisol in AMI is regarded as a stress response. In a previous paper (9) we have shown that, under this circumstance, the central nervous reaction involved in the stress situation seems to override the normal cyclic function of the hypothalamus and the pituitary gland with regard to ACTH release, abolishing the normal diurnal pattern of cortisol secretion.

What factor(s) are of importance in determining the magnitude of the adrenocortical response to AMI is still not clearly understood. Logan and Murdoch (6) found normal levels of hydrocortisone in patients with severe angina pectoris, but with no evidence of myocardial infarction. In this study we were able to demonstrate that the rise in plasma cortisol was paralleled by a rise in venous blood lactate and that there was a significant correlation between peak values for these two parameters. Our data, therefore, suggest that the circulatory failure with a changed metabolism must be considered as a factor in determining the adrenocortical activity. The assumption that the circulatory failure leading to reduced hepatic and renal blood flow, with subsequently reduced removal of cortisol from the plasma, does not appear very likely. It is known that, with an intact and adequately functioning pituitary-adrenal axis, a reduced metabolic removal of cortisol from the blood would immediately result in a reduced adrenal cortisol output.

Logan and Murdoch (6) observed a significant correlation between peak plasma hydrocortisone levels and peak SGOT concentrations. The present study confirms their observation. The cortisol elevation, however, preceded the rise in SGOT.

There has been some controversy about the prognostic value of the plasma cortisol level in patients with AMI. Logan and Murdoch (6) found no significant correlation between a coronary prognostic index taking into consideration factors such as shock, failure and cardiac rhythm, and plasma cortisol level. Their conclusions were supported by Bailey et al. (1), who measured the steroid metabolites in the urine in patients with AMI. On the other hand Klein and Palmer (5) claimed that plasma cortisol above 40 μg/100 ml heralded a grave prognosis, which is supported by our observations. Patients with uncomplicated myocardial infarction had normal or only moderately elevated plasma cortisol. Therefore our study supports the conclusions drawn from previous indirect estimations of the adrenocortical function by counting the blood eosinophils. A prolonged eosinopenia denoted a poor prognosis (2, 3, 4). This method, however, is influenced by other stress mechanisms, such as epinephrine, norepinephrine and the autonomic nervous system (2).

In none of the cases was there evidence of adrenal failure that could be considered a pathogenetic factor in the development of circulatory failure and shock. Thus there seems to be no theoretical basis for the use of exogenous steroid therapy to improve the clinical outcome after myocardial infarction.

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ANXIETY GROWTH HORMONE AND GLUCOSE TOLERANCE IN NORMAL CHILDREN

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Abstract Serum growth hormone and insulin responses to oral and i. glucose have been measured in 13 schoolchildren who had normal but relatively low oral glucose tolerance as judged from preliminary study of total population of 320 schoolchildren. The results were compared with those from 13 children paired for sex, age, eight and height but having average glucose tolerance. Serum growth hormone curves showed an initial peak starting before glucose ingestion in both groups. The rise was significantly higher in oral as well as i. glucose tolerance tests in the group with relatively low tolerance. This group had also tendency to higher serum insulin values. It is suggested that the low carbohydrate tolerance in these children is produced by high growth hormone secretion, elicited by emotional lability

There is little information in the literature about the variance of oral glucose tolerance in normal children (6) and none about the possible correlation between blood sugar response and hormonal responses to glucose administration.

In the present paper a report is given of a *paired* comparison of blood sugar plasma insulin and plasma growth hormone after oral and i.v glucose to *normal children with average and with relatively low carbohydrate tolerance*

In a preliminary field study an oral glucose tolerance test was performed in the 320 schoolchildren of a Danish rural community Fifteen of them, i.e. about 5% were picked out as forming the group of *relatively* low carbohydrate tolerance cases by the arbitrary cut-off point of a blood sugar of 115 mg% 2 hours after drinking the glucose solution Each of these children, whose -hour blood sugar was localized in the treme right part of the distribution curve, was then paired for age, weight, height and sex with an other child whose -hour blood sugar was aver age.

The results of the paired study of these two groups suggest that relatively low carbohydrate tolerance in *normal children* is caused by anxiety leading to increased production of growth hormone.

METHODS AND PROCEDURES

In the preliminary field study it was decided to use *fixed dose* of 50 g glucose, irrespective of the age of the children. This was done so as to avoid the complexities of choosing between dose per kg, per so-called body surface, per 4-hour creatinine excretion or other parameters, and because the apparently lower carbohydrate tolerance naturally to be expected in the smaller children was irrelevant to the study proper in which the comparisons were to be carried out on *pairs*

In the paired test the first blood sample was drawn 15 min before administration of glucose This was done in order to be able to differentiate between the effect of the emotional stress and that of the glucose administration on the growth hormone secretion

The preliminary study included 320 schoolchildren aged 7 17 the entire childhood population of the Farstrup-Lundby parish in Northern Jutland attending the Central School in the village of Farstrup. The 320 tests were performed in the school during one morning. The children and their parents had been instructed about the procedure and the intention of the investigation.

The children arrived at school in the fasting state at 8 a.m. Here they ere divided into 8 subgroups, each sitting in one classroom. Each child as given 50 g glucose in 150 ml ater, and the drinking period as observed to be 1– min Exactly 120 min later ear-blood samples ere collected for blood glucose determination.

Fifteen children had hour blood glucose values f 11 mg% or higher. For the purpose of the detailed study

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Fig. 1. Average blood glucose concentrations during the oral glucose tolerance tests. --- Low tolerance group — average tolerance group.

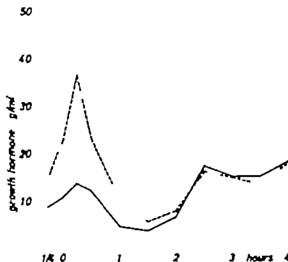


Fig. 3. Average serum growth hormone concentrations during the oral glucose tolerance tests. Symbols as in Fig. 1.

reported in the present paper paired control group was formed by selecting 15 children of similar age and identical sex so that their 2-hour blood glucose values were as near the average for their age group as possible and so that their body eight and height were as near those of their counterparts as possible. Thirteen of these 15 pairs (12 girls and 14 boys) were investigated during a short admission to the Arhus Kommunehospital.

On the second day in hospital 50 g glucose dissolved in 150 ml of water was given orally at 8 a.m. after 10-hour fast. The children had not been allowed to leave their beds during the night. Blood samples were collected from an indwelling venous catheter 15 min before, immediately before, and at 15 30, 45 60, 90, 120, 150, 180 210 and 240 min after administration of glucose.

On the following day glucose was given (50 ml of a solution injected in the course of 4 min). After the fasting blood samples at −15 and 0 min, blood was at 10, 20, 30, 40, 50, 60, 90, 120, 180, 210 and 240 min. These samples were also taken from indwelling venous catheters.

Blood glucose was determined by glucose oxidase method (1), serum insulin and growth hormone were measured radioimmunologically by wick-chromatography technique (5). The *k*-values from the tests were calculated as $k = \ln 2 \quad 100/T \ /$ (2).

Statistical analysis. For calculation of differences we have used the Wilcoxon matched pair signed rank test.

RESULTS

In the sequel the group of children having 2-hour blood glucose values of 115 mg% or higher at the preliminary screening test has been termed relatively low tolerance group and their controls average tolerance group

Oral tests. It is seen from Fig. 1 that the blood glucose values of the average tolerance group are lower than those of the relatively low tolerance group at all points except at $1\,^1/_2$ hours. The statistical analysis showed that the $^1/_2$- ($p =$ 0.026) and 2-hour values ($p = 0.032$) were different. The insulin curves paralleled the blood glucose curves completely and the average tolerance group had lower insulin values throughout the test (Fig. 2). Of the individual time values the 0- and 240 min values were found to be different

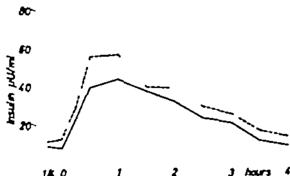


Fig. 2. Average serum insulin concentrations during the oral glucose tolerance tests. Symbols as in Fig. 1

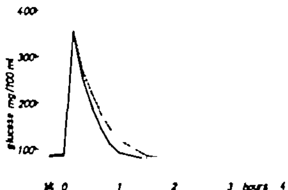


Fig. 4. Average blood glucose concentrations during the glucose tolerance tests. Symbols as in Fig. 1

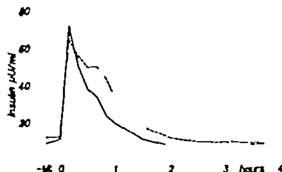


Fig. 3. Average serum insulin concentrations during the I. glucose tolerance tests. Symbols as in Fig. 1

($p = 0.052$) and ($p = 0.042$). The mean growth hormone curves from the two groups (Fig. 3) showed an initial rise which started *before* glucose administration and reached its peak value 15 min after. The mean initial increase of serum growth hormone of the low tolerance group was strikingly larger. The late postprandial rises were however identical. The 0- ($p = 0.052$), 15- ($p = 0.04$), 30- ($p = 0.032$) and 60-min values ($p = 0.026$) were significantly different.

Intravenous tests. It appears from Fig. 4 that the mean blood glucose values of the relatively low tolerance group were higher than those of the average tolerance group during the first 1 $/_2$ hours, i.e. the period when blood glucose was still elevated. It could be shown that the 20- ($p = 0.012$), 30- ($p < 0.001$), 40- ($p = 0.006$), 50- ($p = 0.004$), 60- ($p = 0.006$) and 90-min values ($p = 0.032$) were significantly different.

The *k*-values are given in Table I. In 10 out of 12 pairs the child from the relatively low tolerance group had the lowest *k* value. Statistical analysis gives a *p*-value of 0.006.

As was the case with the oral tolerance test, the serum insulin curves showed higher mean values for the low tolerance group (Fig. 5). Significant differences were demonstrated at 40 ($p = 0.006$), 50 ($p = 0.004$), 60 ($p = 0.026$) and 90 min ($p = 0.054$). The mean growth hormone curves showed the same patterns and differences as in the oral tests, i.e. an initial rise beginning *before* glucose administration, reaching its maximum 10 min after. The secondary rise was again rather similar (Fig. 6). Significant or nearly significant differences were found at 40 ($p = 0.041$) and 30 min ($p = 0.064$).

Table I. *The k-values of the two tolerance groups*

	Low tolerance group		Average tolerance group	
Sex	Age (y.)	*k*-value	Age (y.)	*k*-value
♂	17.8	1.28	16.3	1.16
♂	13.8	1.02	14.8	2.57
♀	13.8	2.04	13.3	3.01
♀	14.0	3.01	16.3	
♂	12.0	1.36	12.0	2.10
♂	11.8	1.47	12.8	1.51
♂	12.0	1.93	10.8	2.17
♀	12.0	2.17	11.8	1.73
♂	11.3	3.30	10.8	3.85
♀	9.0	2.24	8.8	4.07
♀	9.0	2.39	9.3	3.47
♂	7.5	2.67	7.5	4.08
♀	7.5	2.48	7.5	4.07

DISCUSSION

On the basis of the 2-hour blood glucose values of the preliminary screening study we selected for further study the 15 children with values of 115 mg% or higher together with 15 paired controls who had 2-hour values in the average range. Thirteen of these pairs were investigated at the clinic with a renewed 50 g oral glucose tolerance test. In addition a 25 g I. glucose tolerance test was performed. Blood samples were now collected at frequent intervals and analyzed for glucose, serum insulin, and growth hormone.

The group of children who had had a relatively low tolerance at the preliminary test had lower oral tolerance as well as lower k values than the average tolerance group at the renewed tests.

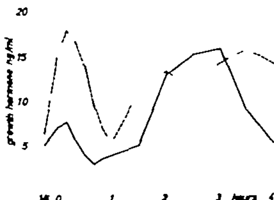


Fig. 6. Average growth hormone concentrations during the glucose tolerance tests. Symbols as in F

The average growth hormone curves showed an initial rise in the oral as well as in the i.v. glucose tolerance test in both groups. The increase was larger in the low tolerance group. The occurrence of a growth hormone release before glucose administration indicates that it was induced by emotional stress brought on by the test situation.

We have thus shown that normal children with a relatively low glucose tolerance have a tendency to higher initial serum growth hormone levels and higher serum insulin values during glucose tolerance tests. This pattern suggests that the relatively low carbohydrate tolerance in some normal children is due to high growth hormone secretion.

It has been known since the introduction of the radioimmunological plasma growth hormone determination that various kinds of stress—pain anxiety fear etc.—stimulate growth hormone release (7). This phenomenon is more pronounced in children than in adults, and is often observed in relation to venipuncture (3, 4, 8). In the present study it was the *initial* growth hormone rise which was more pronounced in the low tolerance group. It seems reasonable to assume, therefore, that the children with relatively low glucose tolerance were characterized by being more emotionally [illegible] or responding more violently to the emotional stress of the test situation.

Finally it should be emphasized that there is no basis for regarding the children characterized by having relatively low carbohydrate tolerance in the present study as being prediabetics in any sense of the word. The prognosis for normal children with relative hypersomatotropism and relatively low carbohydrate tolerance must await further studies in larger groups of subjects.

ACKNOWLEDGEMENTS

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AVAILABILITY OF IRON DEXTRAN FOR HEMOGLOBIN SYNTHESIS

As Studied with Phlebotomy

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Abstract The availability of iron dextran for hemoglobin formation has been studied in six young, healthy male volunteers made anemic and iron depleted by repeated phlebotomy A quantity of 1 850 mg iron dextran was infused calculated to restore not only the Hb deficit but also the iron stores. The rate of Hb production from iron dextran was highest during the first week following infusion and found to be of the same order as in con-trol group in six males receiving iron orally However, from the second eek on, there was significantly slower Hb regeneration in the iron dextran group than in controls, indicating restricted delivery of iron from the iron dextran complex to the erythroid marrow Three to five months after infusion five of the subjects receiving iron dextran ere subjected to new series of phlebotomy in order to quantitate the availability of iron dextran stores for Hb synthesis. When approximately 70% of the calculated iron dextran store of 1 050 mg had been mobilized, an iron deficient erythropoiesis had developed. In this state characteristic residual iron deposits in bone marrow smears could be demonstrated in all subjects.

The i.v infusion of iron dextran (Imferon®) has achieved great interest in the treatment of iron deficiency anemia (1 2, 5 9 14 22, 25, 34 38). In one single i.v infusion an amount of iron can be administered sufficient to restore not only the Hb deficit but also the iron stores. This is usually considered time consuming and difficult by oral iron therapy (8 17 35). The Hb regeneration in subjects treated with i.v iron dextran has been claimed to be faster than with other parenteral iron preparations (37) Iron dextran infusion has also been recommended instead of oral iron in situations where a rapid correction of the anemia is urgent, such as the last trimester of pregnancy (5 9 34) and before surgery (1)

Most clinical reports on the effect of the total dose infusion of iron dextran are concerned with the first few weeks after administration. The findings of stainable iron in bone marrow smears have been taken as signs of adequate iron stores (30 38). However in 1968 Henderson and Hillman (20) showed that the release of iron from the iron dextran complex became progressively restricted with time giving a retarded Hb production in spite of the presence of stainable bone marrow iron. This was in agreement with singular observations of stainable iron deposits in marrow smears of iron deficient subjects previously treated with parenteral iron compounds (3 8 10, 21)

Since one of the main clinical indications for using parenteral iron preparations is the creation of iron stores, it was considered important to get more quantitative information of the utilizability of these artificial iron stores. In a recent study (29) it was found that the iron stores built up by i. iron dextrin (Astrafer® Ferrigen®) was a ail-able for Hb synthesis to approximately 65%

The aim of the present investigation was to study quantitatively the availability of iron stores *created by i. infusion of iron dextran* and to compare the rate of Hb production in anemic subjects, receiving iron dextran infusions, with that achieved with conventional oral iron therapy

The study was performed in healthy male volunteers made anemic and iron depleted by enesection.

SUBJECTS

The subjects of the present study were healthy males, aged 20–33 years. Most of them were regular blood donors since many years, four attended the hospital transfusion service for the first time All subjects were thoroughly informed of the nature of the investigation before they gave their

Table I. *Initial studies and response to iron administration in two groups of subjects made anemic by phlebotomy (means ±1 S.E.)*

Values within parentheses indicate the accumulated increase

	Iron dextran 1850 mg i.v. (n=6)	Oral ferrous succinate–succinic acid 74 mg × 2/day for 4 weeks (n=6)
Initial studies		
Hb before phlebotomy (g %)	14.5±0.3	14.8±0.3
At start of iron administration		
Hb (g %)	9.9±0.[illegible]	10.3±0.3
Serum iron (μg %)	33.8±3.1	77.3±3.0
TIBC (μg %)	461±18	431±15
TIBC satur. (%)	7.4±0.7	6.4±0.7
Blood volume (l)	5.0±0.3	5.1±0.[illegible]
DF-induced iron excretion (mg/24 h)	0.36±0.01	0.35±0.02
Response		
Hb increase (g %)		
1st week	1.5±0.3 (1.5±0.3)	1.7±0.1 (1.7±0.1)
2nd week	0.7±0.1 (2.[illegible]±0.3)	1.4±0.3 (3.1±0.3)
3rd week	0.6±0.1 (2.8±0.2)	0.9±0.[illegible] (3.9±0.3)
4th week	0.7±0.1 (3.5±0.3)	0.7±0.1 (4.6±0.4)

consent. They had no signs of infection, ESR and serum creatinine were normal.

Group I consisted of six male blood donors. Group II consisted of six males, two of whom were regular blood donors, four had no previous blood loss.

METHODS

All blood specimens were taken in the morning by venous puncture with the subject in the recumbent position and in the fasting state. The methods used for the determination of Hb concentration, Hct, serum iron, total iron binding capacity (TIBC), blood volume and total Hb mass, desferrioxamine (DF)-induced urinary iron excretion, were the same as previously described (29). Bone marrow smears were stained for hemosiderin according to the method of Hansen and Weinfeld (1?).

Iron dextran was dissolved in approximately 450 cm³ normal saline and infused within 3 hours. No immediate untoward reactions were noted.

Design of the study

Phlebotomies of approximately 450 ml were performed each week in both groups until iron deficiency and Hb level of approximately 10 g/100 ml were attained (17). The iron deficient state was verified by a low serum iron, saturation of the iron binding capacity of below 10% and a low DF test (Table I). Two to four weeks after the last phlebotomy the blood volume and total Hb mass were determined.

The subjects in group I received 1 850 mg iron dextran in one i.v. infusion. This quantity was calculated to be sufficient to restore the Hb deficit induced by phlebotomy and to build up iron stores of approximately 1 000 mg. The subjects of group II received oral ferrous succinate–succinic acid (Ferromyn® S) corresponding to 74 mg of elemental iron twice daily during four weeks. Repeated blood counts were carried out during these weeks in both groups.

Study of the Hb production rate in iron deficiency anemia

The rate of Hb production between the two groups was compared and expressed as increase in Hb concentration and in Hb synthesized (g). The amount of Hb synthesized was computed from the product: Hb increase (g/100 ml) × blood volume (ml) × 0.87, where 0.87 was the correction factor for venous Hct/body Hct ratio used (4). The amount of iron utilized for Hb synthesis was calculated (1 g Hb contains 3.4 mg iron). The blood volume was assumed to be unchanged during these 4 weeks.

Availability of iron dextran stores for Hb synthesis (Fig. 1)

Five of the volunteers receiving iron dextran were followed regularly after normalization of the Hb level. Three to five months after the infusion of iron dextran the blood volume and total Hb mass were remeasured before the mobilization of iron by a second series of phlebotomies was started. The quantity of iron stored in the body at this time was calculated from the amount of iron dextran

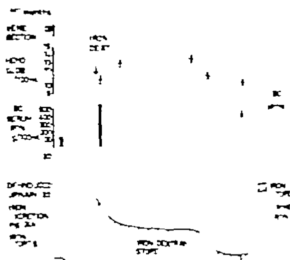


Fig. 1. Five blood donors underwent weekly phlebotomies to iron deficiency anemia. 1 850 mg iron dextran i.v. was then administered. Four months later a new series of phlebotomies was carried out until retarded erythropoiesis was achieved. (See also Table II.)

infused, subtracted by the amount consumed for Hb formation. Iron consumed for Hb synthesis was calculated from the difference between the iron content of the actual Hb mass and the iron content of the Hb mass at the time of iron dextran infusion. The iron content of blood specimens drawn during this period was subtracted. N cor rection was made for iron absorption from food during the interval with repleted iron stores.

Then second series of phlebotomies was started and continued until signs of retarded erythropoiesis were achieved (29). When the Hb value had remained unchanged for -4 weeks after discontinued venesections, the serum iron as low the TIBC elevated and the DF induced iron excretion in urine low it was considered that the available iron stores were depleted (Fig. 1). At this time the blood volume and total Hb mass were again determined, bone marrow aspiration was performed and the smears were examined for stainable iron.

The amount of iron mobilized from the iron stores was calculated as previously described (29) from the iron content of removed blood, corrected for iron content of the Hb deficit and iron absorbed from food (3 mg/day) (10).

RESULTS

Availability of iron dextran for Hb production as compared to oral iron medication in iron deficiency anemia (Figs. 2 and 3 Table I)

The levels of Hb concentration before and after phlebotomy blood volume, serum iron and TIBC were similar for the two groups. The rate of Hb increase was of the same order for the two groups within the first week, but after that time the iron dextran group responded more slowly The quantity of Hb produced from the second to the fourth week was significantly lower ($p < 0.05$) in the iron dextran than in the orally treated group. The amount of iron delivered to the erythroid marrow

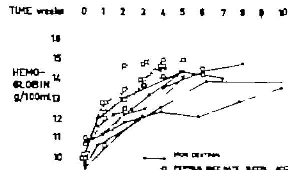


Fig. 2 Rate of Hb increase in six subjects receiving 1 850 mg iron dextran in total dose infusion and in six subjects taking oral ferrous succinate + succinic acid (Ferronyl® S) 74 mg two times daily during four weeks.

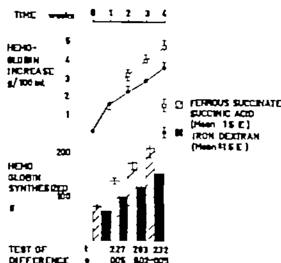


Fig. 3 Hb increase in the two groups studied and the corresponding amount of Hb synthesized. (As seen in Fig. the Hb concentrations in the iron dextran group were not determined exactly at weekly intervals. Hence, in the calculation of Hb production extrapolations from the Hb curves in Fig. 2 were performed.)

required to give this Hb increase averaged 36 mg/day during the first week. However during the following 3 weeks the iron delivery from iron dextran was restricted to approximately 15 mg/day as compared to 24 mg/day for the group receiving oral iron.

Fig 1 and Table II show that during the first week following iron dextran infusion the iron concentration of serum was extremely high. The DF-induced urinary iron excretion averaged 3 15 mg/ 4 h on the fifth day This high value was not due to a spontaneous iron excretion due to the iron dextran infusion, since in subjects not given DF the maximal urinary iron excretion following iron dextran did not exceed 0 3 mg 24 h. The elevated DF value is approximately twice as high as that of normals with iron stores of a corresponding size (28)

The Hb concentration approached normal values 6–8 weeks after iron dextran infusion. The serum iron concentration decreased to subnormal values from 4 weeks following infusion. None of the five subjects continuously followed reached their original serum iron levels. The DF-induced urinary iron excretion averaged 0.80 mg 1–4 months after the infusion. This value was slightly lower than that of normal iron stores of a corresponding size (28)

Table II *Hb level serum iron, TIBC blood volume and DF-induced iron excretion in urine in five blood donors at different stages of the study (means ±1 S.D)*

Stage of experiment	Time after infusion (d.)	Hb (g)	Fe (µg)	TIBC (µg)	Blood volume (l)	Iron store (mg)	DF-induced iron excretion (mg/24 h)
At first phlebotomy		14.2±0.3	115±19	378±48		122±77	0.51±0.04
After phlebotomy at iron dextran infusion	0	9.9±0.5	33±8	456±47	5.1±0.7	0	0.35±0.03
	5	10.8±0.5	>1 200	—			3 15±1.20
	28	12.9±0.4	76±10	348±40			0.79±0.08
	60	13 7±0 8	74±13	321±16			
At start of second series of phlebotomy	120	13.6±0 5	77±20	330±45	5.3±0.5	1 053±114	0.81±0 15
After phlebotomy	188	10.5±0.4	33±5	437±34	5 1±0.6	0	0.39±0 05

Availability of iron stores created by iron dextran for Hb formation (Fig. 1 Tables II and III)

The fraction of the infused iron dextran utilized for Hb synthesis up to 4 months after administration averaged 777 mg. Thus, the remainder 1 053 mg (range 934–1 234), was stored. The availability of this storage iron for Hb synthesis was studied by means of quantitative phlebotomy During the first weeks of phlebotomies the Hb concentration showed a sharp fall. Then the curve levelled off indicating that the Hb production was almost equal to the amount of Hb removed each week. Further venesections induced a progressive de of the Hb concentration and the phlebotomies were discontinued. Since the Hb level then remained unchanged, the saturation of TIBC remained below 10% for 3–4 weeks and the DF induced urinary iron excretion indicated iron deficiency it was considered that the available iron stores were depleted.

Table III shows that the quantity of iron removed from the stores averaged 742 mg or 70.5% (65–78%) of the calculated iron dextran iron stores. Thus, approximately 300 mg were not accounted for

In all subjects residual iron deposits were easily demonstrated in bone marrow smears 2–3 weeks after the last phlebotomy They had a characteristic appearance of small, equal-sized granules located to reticuloendothelial (RE) cells. No sideroblasts were found. The subsequent oral iron administration gave a rapid Hb increase.

DISCUSSION

Several studies in animals and man have pointed out a rather distinct metabolic pathway of administered iron dextran (12, 13 20 33 38). Given i.m. a fraction of approximately 30% is retained in the muscle (11 13). From the plasma compartment the iron colloid is slowly cleared into RE cells, where the complex has to be split before

Table III *Utilization of iron stores built up by iron dextran*

Subject no.	Iron dextran admin. (mg)	Fe used for Hb synthes. (mg)	Fe stored (mg)	Fe content of Hb removed by phlebotomy (mg)	Fe content of Hb deficit (mg)	Fe abs. from food (3 mg/d.) (mg)	Fe mobilized from stores: Corr for Fe abs. (mg)	(% of Fe stored)	Not corr for Fe abs. (mg)	(% of Fe stored)	Stainable iron in bone marrow smears after phlebotomy: Reticular (grade 0–4)	Sideroblast (%)
19	1 800	819	981	1 457	557	204	696	71	900	92	I	0
22	1 850	616	1 234	1 629	600	210	819	66	1 029	83	I	0
23	1 800	732	1 068	1 605	536	234	835	78	1 069	100	I	0
25	1 850	801	1 049	1 505	552	198	755	72	953	90	I–II	0
28	1 850	916	934	1 244	467	168	609	65	777	83	I	0
Mean	1 830	777	1 053	1 488	542	203	742	70	946	90		

iron ions are liberated for transferrin and transported to the erythron (12, 13 20) By graded phlebotomy adjusted to keep a constant Hct level, Henderson and Hillman (20) were able to quantitate the release of iron from the iron dextran to the erythroid marrow Immediately after infusion they found that a small fraction of the iron was removed from the complex and reacted directly with transferrin. However the major part of the complex had to be processed within RE cells before iron was released to plasma transferrin. The release was very rapid during the first few days permitting a high synthesis of Hb. Within 10 days, however the release became progressively restricted in spite of presence of significant iron stores.

The findings in the present study are in agreement with those of Henderson and Hillman. The Hb increase was rapid during the first week following iron dextran infusion as a reflection of large amounts of available iron. The increased urinary iron excretion after DF is also consistent with the presence of abundant available iron. However from the second week the Hb production rate was slower This was not due to limitations of the marrow capacity itself since the control group receiving oral iron continued with a greater production. The reduced response must therefore be due to a restricted delivery of iron to the erythroid marrow The subnormal serum iron values 1–4 months following iron dextran infusion were probably also reflections of this retarded iron release and are in agreement with previous observations of Marchasin and Wallerstein (25) Wood et al. (38) and Henderson and Hillman (20) The availability of iron dextran stores for DF chelation was also slightly reduced during this period as compared to natural iron stores of corresponding size (28). This is in agreement with the findings of Hedenberg (19)

The average rate of Hb increase of 0.9 g/100 ml/week in response to iron dextran is of the same order as in previous reports (1 5 14 16). Will et al (37) found a faster Hb production during the first 2 weeks following infusion. The difference might be due to the fact that the Hb level at the start was lower in their subjects.

It is reasonable to assume a faster rate of Hb synthesis if the single infusion would be replaced by repeated smaller ones. This has been shown by Dawson et al. (9).

However the results of the present study give no support to the opinion that parenteral iron compounds should be recommended in situations where a rapid correction of anemia is urgent. This is in agreement with the results of previous comparative studies between the effects of parenteral iron preparations and oral iron salts (7 26, 31).

The second part of the present study was designed to quantitate the availability for Hb synthesis of the "artificial" iron stores 3–5 months after the infusion of iron dextran. In response to vigorous phlebotomy there was a rapid fall in the Hb concentration, indicating a restricted iron delivery to the marrow and a fraction of 30% of the calculated iron stores was found to be unavailable for Hb production. In spite of the iron deficient state induced, stainable iron deposits were seen in the bone marrow of all subjects studied. The findings correspond well to previous reports (8 10, 20 21) The deposits had the same appearance of small, equal-sized granules in RE cells as described after iron dextrin (29) and sacharated iron oxide (3 18) Thus, there is a difference between these artificial iron stores and normal ones, which are completely available in response to serial phlebotomies of this kind (24 27 28)

Richter (32) found that it is not possible to distinguish injected iron dextran from endogenous hemosiderin by routine histochemical methods. The pattern of deposition of iron dextran stores in the marrow RE cell system has been described as apparently similar to that of normal stores of hemosiderin iron (20, 30). However Hausmann et al. (18) described the picture of small, uniform-sized granules in subjects receiving parenteral iron therapy as a special type of iron storage. In 15 patients relapsing into iron deficiency anemia they found the characteristic iron granules of grade 1+ to 4+ (on a scale 0–6+) still present.

Possible explanations of the restricted availability of high molecular iron complexes have been discussed by Henderson and Hillman (20) and Olsson et al. (29). It might be due to differences in distribution and/or physicochemical character of the material as compared to normal storage iron.

Few studies have been made in man on the distribution of iron dextran within the liver Based

on animal studies it is generally believed that most of the iron is taken up by Kupffer cells. The findings of Block, as cited by Henderson and Hillman (20) are in agreement with this assumption. He found a minimal iron uptake in liver parenchymal cells in man. On the other hand Lundin et al. (23) observed considerable amounts of iron both in Kupffer cells and parenchymal cells as soon as one week after iron dextran infusion. These few observations concerning iron dextran suggest a difference in distribution as compared to that of normal liver iron. In normal man most of the iron is located to parenchymal cells, while stainable iron in Kupffer cells usually is absent (36).

Using immunochemical assay Cook (6) showed that the water soluble fraction of liver iron in three subjects receiving iron dextran infusion was up to 90% ferritin. This is of the same order as in control subjects. The proportion of water soluble iron of the total non-hemin iron content was also of the same magnitude as in controls. In other words, Cook's study did not indicate that the chemical composition of the liver iron in subjects receiving iron dextran differed from that of normal livers. It is, however, not known if the insoluble fraction of non-hemin iron (which is not accessible for immunoassay) is entirely composed of true hemosiderin or if it also contains iron dextran complexes.

At present it is not possible to give a distinct explanation of the incomplete utilizability of iron dextran iron stores. It might be due to different physicochemical properties of a fraction of the material making it difficult to be metabolized within the RE cells. Differences in distribution might also be of importance.

No harmful effects of these residual iron deposits within RE cells have been reported so far (21). However, their presence may spoil the value of the bone marrow stain in the diagnosis of iron deficiency anemia.

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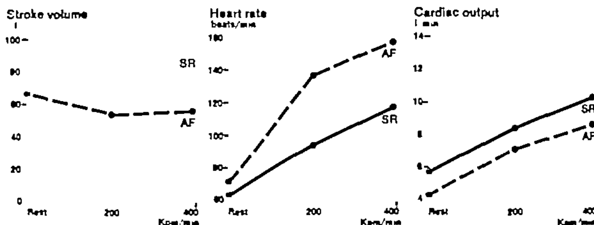
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Stroke vol me heart rate and cardiac output at rest and during e e cise Mean values of the same 7 men during atrial fibrillation and sinus rhythm (3 males with RHD and 4 without RHD).
Cramér G. Acta Mod Scand suppl. 490 1968.

Cardiac output is increased, enabling the patient to lead a more active life

Without maintenance treatment the risk of recurrence of fibrillation is great

Of the available anti-arrhythmic agents, **quinidine** is the best documented drug in maintenance therapy after cardioversion of atrial fibrillation

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AVAILABILITY OF IRON SORBITOL FOR HEMOGLOBIN FORMATION

As Studied with Phlebotomy

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Abstract. The vailability of iron sorbitol for hemoglobin synthesis has been studied in six young, healthy male *volunteers made anemic by repeated venesections.* The rate of Hb production from iron sorbitol was compared to that after oral iron therapy and was found to be of the same order except for the first eek of treatment, when iron sorbitol gave slower response. This delay was thought to be due to processing of the iron sorbitol complex before iron could be delivered to the erythroid marrow In another group of six male volunteers, made iron deficient by phlebotomy iron stores are created by iron sorbitol of size as encountered in normal men The availability of these iron stores for Hb production was studied by means of quantitative phlebotomy The amount of iron mobilized from the iron sorbitol stores averaged 94% of the calculated store of 727 mg No significant residual iron deposits could be demonstrated after completed venesections. The urinary iron excretion after desferrioxamine (DF) in subjects treated with iron sorbitol was found to be significantly higher than in subjects with normal iron stores of corresponding size. It was suggested that this was due to localization of fraction of iron sorbitol to tubular cells of the kidneys where DF reaches high concentration.

Some previous observations indicate that iron stores built up by parenteral iron preparations, such a saccharated iron oxide iron dextrin and iron dextran, are not completely a ailable for Hb synthesis (3 5 8 10 11) Henderson and Hillman (11) showed that the delivery of iron from stored iron dextran became more and more restricted with time. Quantitative studies from this laboratory on the availability of iron dextrin and iron dextran stores showed that a fraction of about 30–40% was not available for Hb synthesis (17 18)

It has been proposed that this unavailability might be due t the physical character of the high molecular compounds, which makes them difficult to dissolve within the reticuloendothelial (RE) cell system (11). The iron sorbitol-citric acid comple (Jectofer[1]) differs from these high molecular compounds by a mean molecular size of about 5000 (12) This implies a different distribution within the body and it has been proposed that the RE cell uptake of iron sorbitol is minimal (7)

The utilizability of the iron sorbitol complex in iron deficient subjects is well documented (1 7 14 20 21) However no studies have been per formed in order to test if the iron stores built up in this way are completely a ailable for Hb production. As one of the main clinical indications for using parenteral iron preparations is the creation of iron stores, it is of great clinical interest to test if this storage iron can be used if need arises.

The aim of the present investigation was to test the availability of iron sorbitol for Hb synthesis. The studies were performed in healthy male volunteers made anemic and iron deficient by phlebotomy

The utilizability of iron sorbitol was first studied in the anemic situation. In another group the availability of iron sorbitol stored for 3–4 months was studied by means of quantitative phlebotomy The chelatability of the iron sorbitol stores for desferrioxamine (DF) was repeatedly studied dur ing the investigation.

SUBJECTS

The subjects of the present study ere healthy males, aged 21 33 years. Most of them ere regular blood donors for many years, few attended the hospital transfusion service for the first time. After detailed information they were asked to participate as paid volunteers. They

had no signs of infection, ESR and serum creatinine were normal, no proteinuria was present.

Group I consisted of six males, four regular blood donors, two had never given blood before. Group II consisted of six males, two blood donors since 5 years, four had never given blood before. Data on these subjects have been presented previously (18). Group III consisted of six males, five regular blood donors, 1 had never donated blood before.

METHODS

All blood specimens were taken in the morning by venous puncture with the subject in the recumbent position and in the fasting state. The methods used for the determination of Hb concentration, red cells, Hct, serum iron, total iron binding capacity (TIBC), blood volume and total Hb mass, DF-induced urinary iron excretion and the mobilizable iron stores were the same as previously described (17). Bone marrow smears were stained for hemosiderin according to the method of Hansen and Weinfeld (9). Iron in urine, collected before DF administration, was determined after wet ashing using orthophenanthroline.

To get the exact amount of iron sorbitol administered, the ampoules and syringes were washed in saline after the injections. The iron content of the wash was analysed by wet ashing and subtracted from the total iron content in each ampoule of the actual batch according to the manufacturer.

Availability of iron sorbitol for Hb synthesis during anemia

The subjects in groups I and II were phlebotomized (450 ml blood each week) to iron deficiency anemia with [illegible] concentration of approximately 10.5 g/100 ml (15). The iron deficient state was verified by low serum iron, elevated TIBC and low DF test. [illegible]–4 weeks after the last phlebotomy the blood volume and total Hb mass were determined. The subjects in group I were then administered iron sorbitol 100 mg/day 5 days/week to a total amount of 1 700 mg within [illegible] weeks. This dose was calculated just sufficient to restore the Hb deficit induced by phlebotomy. Repeat blood counts were then carried out at weekly intervals. Iron lost in urine after iron sorbitol injections was determined from at least 3 different 24-hour urine analyses. The amount of iron sorbitol administered − iron lost in urine = iron sorbitol left for Hb synthesis.

Group II obtained ferrous succinate + succinic acid (Ferromyn® S), corresponding to 75 mg iron twice a day during 4 weeks, and the Hb increase was followed.

The amount of Hb produced during 4 weeks = Hb mass at 4 weeks − Hb mass at start + Hb in blood samples removed during the period. Hb produced (g) × 3.4 = mg of iron used for Hb synthesis. The total blood volume was considered to be the same during the 4 weeks as that determined before the start of iron administration. A correction factor of 0.87 for the venous body Hct ratio was used (16).

Thus, iron (mg) used for Hb formation during 4 weeks = Hb increase (g/100 ml) × blood volume (ml) × 0.87 × 3.4.

The rate of Hb formation from iron sorbitol was compared to that of the group receiving iron orally.

Availability of iron stores built up by iron sorbitol

The six healthy male volunteers in group III underwent weekly venesections to iron deficiency anemia as previously described. Their iron deficient state was verified by low serum iron, elevated TIBC and low DF test. Blood volume and total Hb mass were determined 3 weeks after the last phlebotomy.

The purpose was now to create iron stores of approximately 1 000 mg by iron sorbitol injections, 100 mg/day. As 35% of the administered iron would be lost in urine and a fraction would be used for Hb synthesis, the creation of these iron stores would necessitate a great number of injections. A pilot study showed that it would be difficult to give more than 20–25 iron sorbitol injections to these subjects. To ascertain that most of the administered iron would be placed in the stores the initial experimental design (18) had to be modified. In order to reduce the amount of iron to be used up for Hb synthesis the grade of anemia after phlebotomy was reduced by oral iron administration. A low dose of oral iron, corresponding to 75 mg elemental iron/day was therefore given for 2 weeks. Thereafter the Hb concentration remained unchanged. As serum iron and transferrin saturation also were still low it was considered that all oral iron absorbed had been used for Hb synthesis and no iron had been stored. In this state of moderate iron deficiency anemia the subjects were given a series of iron sorbitol injections of 100 mg/day, total dose 2 100 mg (range 1 900–2 600). During the 24 hours following an iron injection the urine was collected in iron free polyethylene bottles. 10% of each 24-hour urine portion was pooled and kept frozen until iron analysis. The urinary sediment was checked up weekly during each series of iron sorbitol administration. The Hb concentration, serum iron and TIBC were followed regularly during 5 months up to the start of the second series of phlebotomy when the total Hb mass and blood volume were remeasured. The amount of iron stored at this time was calculated as follows: iron sorbitol administered − iron lost in urine = iron left in the body. Iron left in the body − iron used for Hb synthesis = stored iron. Iron used for Hb synthesis = iron content in Hb mass at 5 months − iron in Hb mass at start + iron content in blood removed during that interval.

The purpose of the second series of phlebotomy was to evaluate if the iron stores built up by iron sorbitol were available for Hb formation. The subjects underwent weekly venesections until iron deficiency anemia was achieved as previously described (17). When the TIBC saturation remained lower than 10% for 5–6 weeks it was considered that no more iron was delivered from the stores to the erythroid marrow. At this time a bone marrow aspiration was performed and stained for iron, and the blood volume and total Hb mass were remeasured.

The amount of iron mobilized from the stores was calculated as previously described (17) and expressed as per centage of the amount of iron sorbitol stored.

The DF-induced urinary iron excretion was repeatedly studied throughout the investigation.

Table I. *Initial studies and response to iron administration in two groups of subjects made anemic by phlebotomy (means ± 1 S.E.)*

Values within parentheses indicate the accumulated increase

	Iron sorbitol i. m. (= 6)	Oral ferrous succinate + succinic acid (= 6)
Initial studies		
Hb before phlebotomy (g %)	14.9 ± 0.2	14.8 ± 0.3
At start of iron administration		
Hb (g %)	10.5 ± 0.3	10.3 ± 0 3
Serum iron (µg %)	27.3 ± 2.5	27.3 ± 3 0
TIBC (µg %)	412 ± 14	431 ± 15
TIBC saturation (%)	6 6 ± 0 5	6 4 ± 0.7
Blood volume (l)	5.2 ± 0.2	5.1 ± 0 2
Response		
Hb increase (g %)		
1st week	0 9 ± 0.1 (0.9 ± 0 1)	1 7 ± 0.1 (1 7 ± 0 1)
2nd week	1 4 ± 0.3 (2.3 ± 0.3)	1.4 ± 0.3 (3.1 ± 0.3)
3rd week	1 1 ± 0.2 (3.4 ± 0.1)	0.9 ± 0.2 (3.9 ± 0.3)
4th week	0.4 ± 0.2 (3.8 ± 0.3)	0.7 ± 0.1 (4 6 ± 0.4)

RESULTS

Availability of iron sorbitol for Hb synthesis during anemia

The results are summarized in Tables I and II and Fig. 1 The initial level of Hb concentration, blood volume serum iron and % saturation of the iron binding capacity were similar for the two groups. The mean increase in Hb concentration

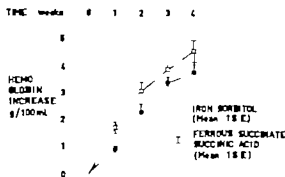


Fig. 1. Response to iron administration in tw groups of iron deficient subjects, one receiving iron sorbitol i.m. 100 mg/day 5 days week during 2 weeks, the other receiving iron orally as ferrous succinate + succinic acid corresponding to 74 mg 2/day of elemental iron during 4 weeks

after one week was significantly lower ($p < 0.005$) in the iron sorbitol group than in the orally treated. From the second to the third week the Hb production was the same for the two groups. During the fourth week, when the iron sorbitol administration had been discontinued Hb increase was again slower than in the group treated with oral iron.

The quantity of iron delivered to the erythroid marrow during the first week, necessary to give this rate of Hb increase averaged 20 mg day in the iron sorbitol group as compared to 34 mg/day in the group receiving oral iron During the following 2 weeks the corresponding iron delivery averaged 7 mg/day for iron sorbitol and 25 mg/day for ferrous succinate.

Table II. *Utilization of 1200 mg iron sorbitol for Hb formation within 4 weeks*

	At start							At 4 weeks						
									Iron used for Hb synthesis			Urine iron	
Subject no.	Blood volume (l)	Hb (g %)	Urine iron after DF (mg/24 h)	Iron sorbitol admin. (mg)	Iron lost in urine (%)	Iron lost in urine (mg)	Iron sorbitol retained (mg)	Hb (g %)	(mg)	(% of iron sorbitol admin.)	Iron sorbitol stored (mg)	Before DF (mg/24 h)	After DF (mg/24 h)
10	5.3	10.1	0.53	1 200	37.5	450	750	13 1	535	46	195		1.03
17	5 0	9.8	0.27	1 200	37.6	451	749	13.7	624	52	125	0.04	0 58
21	5 8	10 1	0.48	1 200	37 4	449	751	13 9	714	60	37	0.08	1 14
24	5 4	10.8	0.41	1 200	38.5	462	738	14.3	623	52	115	0 06	1.10
29	4.5	10.5	0 42	1 200	34.0	408	792	15.6	765	64	28		
30	5.4	11.8	0 44	1 200	38.8	466	734	15.0	584	49	150	0 05	2.04
Mean	5 2	10.5	0.43	1 200	37.3	448	752	14.3	644	54	108	0.06	1.2
S D	0.4	0.7	0.1	0	1 7	21	21	0.9	80	6.8	65	0 02	0.5
S.E.	0.2	0.3	0 04	0	0.7	8.4	8.4	0 4	33	2.8	27	0.01	0.24



Fig Results of t o series of quantitative phlebotomy in subject 11

The mean iron loss in urine within 24 h after iron sorbitol injection was 37.3 The fraction of the administered iron sorbitol, used for Hb synthe sis within 4 weeks (10 days after discontinued iron administration) averaged 54% Thus, approx imately 9 were not directly accounted for The DF-induced urinary iron excretion at 4 weeks a eraged 1 mg 4 h, a alue higher than that of normal males (15)

ailability of iron stores built up by iron sorbitol

shows the experimental design and the re ults of phlebotomy in subject 11 He was a healthy medical student and had never donated blood before entering this study

In response to frequent venesections he de eloped a mild iron deficiency anemia verified by bone marrow examination a low urinary iron ex cretion after DF and a TIBC saturation of below 10% At that time 820 mg iron had been mobilized from his depots. Corresponding amounts for the other subjects of the group ranged from 0 to 207 mg (15) After a short course of oral iron medication the Hb concentration was controlled for 2 weeks. As the Hb level was unchanged and the TIBC saturation still low it was considered that no storage iron was present. In this state of moderate iron deficiency anemia a series of iron sorbitol injections was administered. He was given 1 920 mg iron sorbitol. 32.4% or 622 mg was lost in urine and 430 mg used for Hb formation during the following weeks. Thus 868 mg was left in the stores. In response to a new series of weekly phlebotomies he could mo-

Table III. *Utilization of iron stores built up by sorbitol for Hb synthesis*

Subject no.	Iron sorbitol admin. (mg)	F excret. in urine (mg)	F excret. in urine ( )	F used for Hb synthes. (mg)	F stored (mg)	F content of Hb removed by phlebotomy (mg)	F content of Hb deficit (mg)	F bsorb from food (3 mg d) (mg)	Fe mobilized from stores: Corr for Fe bsorb. (mg)	Fe mobilized from stores: Corr for Fe bsorb. (of F stored)	Fe mobilized from stores: Not corr for F bsorb. (mg)	Fe mobilized from stores: Not corr for F bsorb. (of Fe stored)	Stainable reticular iron in bone marrow smears after phlebotomy
11	1 920	622	32.4	430	868	1 747	792	19	736	85	955	110	0
21	010	687	34.2	500	823	1 887	723	231	933	113	1 164	141	0
4	2 110	777	36.9	653	680	1 728	996	10	522	77	732	108	Trace
79	2 020	689	34.1	491	840	1 794	449	19	976	110	1 145	136	0
30	2 030	745	38.2	923	362	1 708	1 117	198	393	108	591	164	Trace
37	620	1 022	39.1	805	793	1 545	744	228	573	72	801	101	0
Mean	118	757	35.8	634	727	1 735	837	218	681	94	897	126	

Table IV *Hb level, mean corpuscular volume (MCV), serum iron TIBC, TIBC saturation ., blood volume and urinary iron excretion in six male blood donor volunteers (means ± 1 S.D)*

The subjects first underwent eekly venesections to depleted iron stores and anemia (*A*). A small dose of oral iron was thereafter administered for 14 days. Hb was then controlled for 14 day and iron sorbitol administered (*B*). The second series of phlebotomy began 3–6 months later (at *C*). When no more iron could be mobilized from the stores the situation described at *D* had developed. See also Fig. 2

	Hb (g %)	MCV (μ^3)	Fe/s (μg %)	TIBC (μg %)	TIBC satur (%)	Blood olume (l)	Actual iron store (mg)	Urinary iron Spontaneous (mg/24 h)	After DF (mg/24 h)
A	10.4±0.3	84±5	30± 9	420±51	7 4±2.2	5.3±0.7	0	—	0.36±0 04
B	12.4±0.8	84±4	44±11	409±52	11.0±3.2	(5.3±0.7)	0	—	
C	14.7±0.7	90±3	131±23	356±31	37.1±8.5	5.6±0.2	727±191	0.11±0.0	1 13±0.2
D	10.3±0.5	84±4	27± 6	410±31	6.7±1.6	5.3±0 6	0	0.06±0 02	0 47±0.06

bilize 792 mg or 85% of the calculated iron sorbitol store. No stainable iron deposits could be detected in bone marrow smears after phlebotomy

Table III shows the results for the group. Of the total amount of 2 118 mg iron sorbitol administered, the urinary iron loss averaged 35 8% or 757 mg. The quantity of iron mobilized from the iron sorbitol stores averaged 94% of the calculated stores of 727 mg. In two subjects trace amounts of stainable reticular iron could be detected after phlebotomy In four subjects no stainable iron was demonstrated.

Table IV shows the changes in parameters studied during the experiment.

DF chelatability of iron stores built up by iron sorbitol

Table II shows that the DF-induced urinary iron excretion averaged 1.2 mg/24 h 10 days after discontinued iron sorbitol administration and at a time when the iron stores were smaller than 200 mg. The spontaneous iron excretion before DF fell within normal limits (Table V)

Results of repeated DF tests in subject 11 are shown in Fig. . The DF-induced urinary iron excretion was increased when his normal iron store was replaced by an equal-sized one created by iron sorbitol. It can also be observed in Fig 3 that there was an almost linear decrease in the DF-induced urinary iron excretion repeatedly studied during the mobilization of the iron sorbitol stores.

The relationship between urine iron after DF and the iron stores of seven blood donors who –6 months earlier had been gi en iron sorbitol is seen in Fig. 4 All subjects except one fell outside the normal range. He had from the beginning excreted lower iron amounts in the urine after DF than expected. He was apparently healthy had no proteinuria, urinary sediments were normal and serum creatinine 1.2 mg 100 ml He had, however a somewhat elev ted BP (170 100) and was found to ha e a reduced capacity to concentrate the urine. Hence, it is possible that the lowered urinary iron excretion observed after all DF injections was a consequence of tubular damage.

Thus, iron stores built up by iron sorbitol differ from normal ones by a significantly higher DF-induced urinary iron excretion.

Table V *Urinary iron excretion (mg/24 h) in male subjects receiving iron sorbitol i.m. as compared to the "spontaneous" iron excretion of a group of male controls (means ± 1 S.D)*

After 100 mg iron sorbitol 0–24 h	24–48 h	48–72 h	72–96 h	10 d. after completed admin. of 1200 mg iron sorbitol	3 mo after completed admin. of 2100 mg iron sorbitol	After phlebot reduced deplet. of iron sorbitol stores	"Spontaneous iron excretion in male controls
n = 13	n = 5	n = 3	n = 1	n = 7	n = 6	n = 5	18
36.9±3.3	1.5±0.7	0.34±0.11	0 10	0.07±0 03	0.11±0 0	0 06±0 02	0 05±0.03

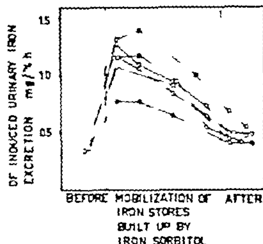


Fig 3 Result of repeated measurements of the urinary iron after DF in the six subjects of the study Note the almost linear reduction of the urinary iron excretion after DF during the period of phlebotomy The DF values after phlebotomy did not reach quite the same low values as at the start of the experiment.

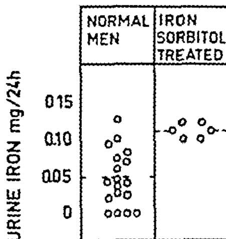


Fig 5 Spontaneous iron excretion in urine during 24 hours in two groups of subjects, 18 men with normal iron stores as measured by the DF test and 6 blood donors with iron stores built up by administration of 100 mg iron sorbitol 4 months ago.

Fig. 5 and Table V show that the spontaneous iron excretion in urine also after 4 months following administration of 100 mg iron sorbitol is significantly higher than that of a male control group. However after depletion of the iron sorbitol stores by phlebotomy the spontaneous iron excretion in these subjects fell to values within limits.

The DF-induced urinary iron excretion after this second series of phlebotomy averaged 0.47 ± 0.06 mg 24 h and indicates iron deficiency However this value is significantly higher than that after the first series of phlebotomy (0.36 ± 0.04 mg 24 h)

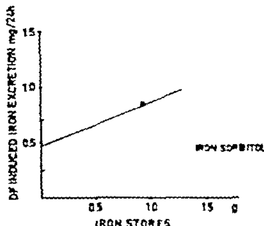


Fig 4 Relationship between DF-induced iron excretion and the iron stores created by iron sorbitol in seven subjects. The regression line and 95% confidence limits of DF-induced urinary iron to mobilizable iron stores in normal males from previous study 15) are depicted.

DISCUSSION

The first part of the study was designed to test the rate at which iron from the iron sorbitol complex is delivered to the erythroid marrow During the first week it was significantly slower in the iron sorbitol group than in that receiving oral iron. This delayed response might indicate that a fraction of the iron complex has to be processed before iron is released to the erythroid marrow After this initial delay the delivery of iron from iron sorbitol was of the same order as that from the intestinal mucosa of the orally treated subjects

It has previously been suggested that iron sorbitol may contain all or much of its iron in a form directly utilizable by the marrow without previous metabolism (7). The present results do not support that suggestion but are in agreement with those reported by Lindvall and Andersson (12) They reported that iron sorbitol contains one dialysable fraction which reacts with transferrin and is immediately available for erythropoiesis, and that the other fraction is taken up by the reticuloendothelial system for further metabolism

The increase in Hb concentration in subjects receiving iron sorbitol averaged 1 1 g/100 ml during the first 3 weeks. This is of the same order as in previous clinical reports on iron sorbitol (1 7 14, 21).

Of the total amount of iron administered the iron loss in urine averaged 37.3% and out of the remainder 85% was used for Hb synthesis within 4 weeks. The amount of iron not accounted for averaged 108 mg. This iron was stored in a form highly available for DF chelation (Table II) This will be *discussed further*

The second part of the experiment was designed to evaluate if iron *stores* created by iron sorbitol were available for Hb formation. In response to frequent venesections there was a rapid release of iron from the storage sites to the erythroid marrow The quantity of iron mobilized from the iron sorbitol stores averaged 94% of the calculated iron stores of 727 mg. However there was a wide range of utilizability in the group. One possible explanation of these individual variations might be the differences in their capacity to absorb iron from food. This is a source of error difficult to correct. During the period of intensive erythropoiesis stimulated by venesections a correction was made for an estimated daily absorption of 3 mg iron from food (8). This figure might have been too high for some and too low for some subjects of the present group.

The present figure of an average utilizability of 94% of the iron sorbitol store suggests a difference from our previous results on the availability of iron dextrin and iron dextran (17 18). Iron stores built up by these high molecular compounds were available to 60–70% for Hb synthesis. However the design of the present study differs from that of our previous studies on iron dextran and iron dextrin as the iron stores built up by iron sorbitol were smaller than the stores previously studied

Nevertheless, the present results show that iron stores built up by iron sorbitol to a size comparable to those of normal men (15) are available for Hb formation in response to phlebotomy No significant residual iron deposits are left in the bone marrow

Previous studies have shown that there is a close correlation between the available iron stores and the urinary iron excretion after DF (2, 13 15)

The present study shows that the urinary iron excretion after DF was significantly higher in subjects receiving iron sorbitol than in normal subjects with iron stores of a similar size. The increased urinary iron content after DF could not be explained by a prolonged spontaneous iron excretion after iron sorbitol administration. As seen in Table V the iron excretion after the first days following iron injections was small

The increased affinity of iron sorbitol stores for DF chelation is probably due to different distribution and/or physical or chemical character of the material as compared to normal storage iron Previous studies in this laboratory showed that the iron stores created by high molecular iron dextrin differed from natural ones by a decreased DF chelatability (17) It was suggested that this was due to the localization of the material to the RE cells, where the release of iron to DF and transferrin was restricted

Animal studies have shown that the ferritin synthesis of the liver is greatly increased after iron sorbitol (22, 23). Wöhler (23) found that this increase was more pronounced after iron sorbitol than after saccharated iron oxide According to Cumming et al. (4) the major source of the iron excreted after DF is liver ferritin iron It might therefore be possible that the increased urinary iron excretion after DF is due to chelation of an increased fraction of liver ferritin in subjects receiving iron sorbitol. However no studies have been made in man that could verify this hypothesis

Another possible target site for the DF chelation in iron sorbitol treated subjects is the proximal tubule of the kidney Wöhler (23) observed a very high concentration of iron in the tubular epithelial cells. Enerbäck and Lundin (6) described the distribution and character of this iron in rats receiving iron sorbitol. They found finely granulated iron in the proximal tubular cells. Electron microscopy suggested that this tubular iron was transformed to ferritin. The rate of disappearance of such iron deposits was higher in anemic animals, indicating that some of the iron could be utilized for Hb production. Studies by Peters et al. (19) show that DF reaches the kidney rapidly after administration. They found a rapid excretion of DF which was due to glomerular filtration but also to active tubular secretion in some species. It is therefore reasonable to assume

that the hyperchelatable pool of iron in subjects receiving iron sorbitol is situated in the proximal tubules of the kidney where DF reaches a high concentration.

The present study also indicates that tubular sorbitol iron can be used for Hb synthesis. The small but significant increase in spontaneous renal iron excretion in subjects receiving 2 100 mg iron sorbitol fell to normal values when the iron sorbitol stores were depleted by venesections. There was also a parallel, linear reduction in the increased DF-induced urinary iron excretion during the mobilization of the iron stores built up by iron sorbitol.

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CARDIOVASCULAR COMPLICATIONS IN MALIGNANT THYMOMA

With Remarks on Pulmonary Hypertension

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Abstract. A case of malignant thymoma with multiple cardiovascular complications is described, and clinical and hemodynamic features, in particular pulmonary hypertension, are briefly discussed in relation to the pertinent literature.

External constriction of the pulmonary artery has been reported in tuberculosis and bronchial carcinoma (9), in at least 36 cases of aortic aneurysm (10), 5 of pericardial band (3), 2 of benign (9, 15) and 2 of malignant (8, 17) mediastinal teratoma, 1 case each of pericardial mesothelioma (20), sternal chondrosarcoma (5), mediastinal tumour of malignant lymphogranulomatosis (9) and lymphoblastoma (2), and in 1 case of benign (19) and 3 cases of malignant mediastinal thymoma (11, 16, 22). Compression of the superior vena cava is rather common in mediastinal tumours (16), also in thymoma (11, 18), which once has been reported to compress the ascending aorta too (11) and not very rarely may infiltrate the pericardium and myocardium (14) and the pulmonary parenchyma (11, 16, 18).

Reported here is a case of malignant thymoma with invasion of the heart and pulmonary parenchyma, compression of the superior vena cava and of the left main and upper lobe pulmonary arteries, general narrowing of the left peripheral pulmonary arteries and angiographic indications of compression of left pulmonary veins. Marked pulmonary hypertension was present, the origin of which is briefly discussed.

CASE REPORT

Male, born in 1917. In Jan. 1961 he had a cylindrical exudative pleurisy. ... chest X-ray also showed ... well demarcated thickening of the left lateral pleura (Fig. 1) which persisted at renewed examinations in Feb. and March 1961.

The patient failed to appear at recommended further examinations. He felt well until Oct. 196 , when burning, lower left chest pains subacutely developed. Chest X-ray now revealed polycyclical leftsided pleural condensations inferolaterally and also anteriorly and along the mediastinal border. A pleural mesothelioma was suspected. After an initial improvement the further course was characterized by rapid progression of the radiological changes, which, although there was also a growing hilar mass, remained consistent with a primary pleural malignancy (Fig. 1 *b*), and of the clinical picture with increasing fatigue, weight reduction, and recurring leftsided chest pains. No certain myasthenic symptoms were recorded.

In Feb. 1966 hoarseness developed, and a leftsided recurrent paralysis was verified in Sept. of the same year. In July ankle swelling, exertional dyspnea and tachycardia started, and in Oct. moderate hepatomegaly was detected. Other physical findings included upper venous congestion and split second heart sound with accentuated pulmonic component.

Laboratory values were unremarkable, except for increased ESR, serum electrophoresis showing an inflammatory reaction but normal γ-globulin fractions, and BSP retention 30%. There were no hematological abnormalities apart from slight neutrophil leucocytosis. Detailed investigations including pleural biopsy, bronchoscopy, bronchography and cytological examination of pleural exudate and sputum did not reveal the nature of the malignant process.

Heart catheterization on Oct. 31, 1966, indicated constriction of the superior vena cava and moderate pulmonary hypertension (Table I). Spirometry at this time showed VC 55% of calculated normal value, TLC 70%, FRC 79%, RV 101%, MVV 63%, FEV 63%, PaO 70 mmHg and PaCO$_2$ 35 mmHg; in the ECG there were generalized T wave inversions in the precordial leads and right axial position and P sinistro-cardiale in lead V1 (Fig. 2). Angiography demonstrated obstruction of the superior vena cava (Fig. 3), compression of the left main and upper lobe pulmonary arteries and general narrowing of the left peripheral pulmonary arteries (Fig. 3 *b*). Th

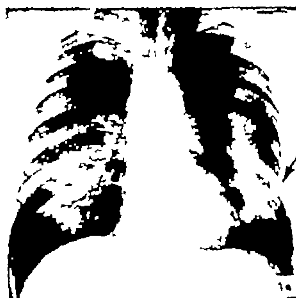




Fig 1 (a) Frontal chest X-ray in Jan. 1961 Well demarcated thickening of the left lateral pleura (arrow). (b) Frontal chest X-ray in Dec. 1966. Extensive tumour growth in the left lung field and hilus.

an evidently slower contrast passage than on the right side and sparse and delayed left pulmonary venous filling with irregularities in the contours of central venous trunks suggesting compression (Fig. 3). In Dec. 1966 explorative thoracotomy was performed via sternum split and revealed massively disseminated, inoperable tumour growth in the mediastinum. Pathoanatomical investigation gave the diagnosis of lymphoepithelial thymoma (Fig 4). In spite of vigorous radiological cobalt treatment with marked clearing of the left lung field lesions and a symptomatological regimen including digitalis and diuretics, the patient cachexia and cardiopulmonary symptoms progressed 1 the early spring of 1967 dysphagia and irritating cough also started. He died on April 12, 1967 in the picture of advanced malignancy and predominantly rightsided cardiac failure.

Table I. *Pressure (mmHg) and flow data and pressure curves from the right atrium and right ventricle*

The pressure curves indicate "heart constriction which can be seen in myocardial as well as in pericardial disease, although more typically in the latter Note equality of the pressure of the right ventricular end-diastolic plateau and the right trial a and wav pressures

Superior vena cava		upper part	17
Inferior vena cava			1
Right atrium		"a wave"	11
		"x"	23
		"v wave"	11
		"y"	15
		mean	7
Right ventricle		systolic	40
	initial	diastolic	1
	end	diastolic	12
Pulmonary artery		systolic	35
		diastolic	10
		mean	22
"PCV"		mean	10
Cardiac output		(l/min)	6
"PVR"		(index)	3

20 mm Hg

40 mm Hg

Pathoanatomical findings

The anterior mediastinum was filled with a large greyish-white tumour mass, growing into the pericardium which could not be loosened from the epicardium (Fig. 5). The superior ena cava and the pulmonary arteries and veins

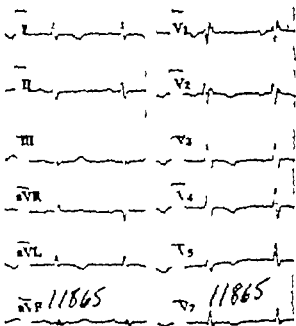


Fig 2 ECG in 1966. Generalized T wave inversions in the precordial leads, rSR configuration and inversion of the P wave in lead V 1







Fig. 3 Angiographic findings. (*a*) Contrast injection in the left subclavian vein, demonstrating complete obstruction of the communication with the superior vena cava, and increased thoracic and neck vein collateral flow. (*b*) Right ventriculography arterial phase. With difficulty the catheter could be passed into the right heart via the right subclavian entry. Note compression of the left main pulmonary artery, delayed contrast filling in the left peripheral pulmonary arteries, which wer narrow par ticularly in the upper and lateral left lung field. ( ) Right ventriculography enous phase. Contrast still remaining in left peripheral pulmonary arteries, pulmonary veins normal on the right side, but very narrow and irregular and sparsely filled on the left side.

w re embedded in the tumour hich do t p he intima of the essels, however Tumour ing om the pleural surface was present in the lef lung lobe. No distant metastases were found in the p lmonary parenchyma. The left pleura as fib ously ob te ed The macroscopic picture of the media tin tumour dif fered considerably from that of the pre ious b p and was dominated by trabecles of rather monom rphous epithelial cells (Fig. 4 *b*) Pronounced dege n changes in terms of fibrosis and organized hemorrh ge er also seen. The pericardium and pleura showed h a fibrosis and sparse infiltration of tumour cells. In the m ocardium, slender streaks of neoplastic cells were seen between the muscle bundles (Fig. 4 *b*) Both lungs xhibited edema and bronchopneumonic changes, and the left lower lung lobe showed fibrosis and tumour infiltration from the pleural surface. No tumour ingrowth into pulmonary es sel lumina could be traced, and there were neither macroscopic nor microscopic changes of pulmonary arteriosclerosis. In the alveoli there was moderate amount of hemosiderin-containing macrophages.

The pathoanatomical findings in other internal organs ere largely unremarkable, apart from signs of chronic venous congestion in the liver and the spleen. No distant metastases were found. The bone marrow and the lymphatic apparatus had normal microscopic appearance, and the skeletal musculature exhibited slight, unspecific atrophic changes.

DISCUSSION

Clinical picture There are no reliable histological criteria distinguishing between clinically benign and malignant thymoma, and therefore the latter differs from the former mainly in more excessive and since distant spread is notably rare locally



Fig. 4. Histological appearance of the tumour. ( ) At biopsy. Epithelial tumour cells surrounded by many lymphocytes. Hematoxylin and eosin, 14. (*b*) At autopsy. Trabeculae of epithelial cells with scant admixture of lymphocytes infiltrating perivascular connective tissue of the myocardium. Hematoxylin and eosin, 100.

infiltrative growth (18). It is not unusual that a malignant thymoma, particularly of the "granulomatous" type (18), may develop predominantly over a lung field rather than in the mediastinum (11, 16, 18) and thereby mimic a pleural malignancy. Chest pains (11), recurrent nerve paralysis and compression of esophagus and trachea (18) may all belong to the clinical manifestations of the tumour.

Immunological abnormalities, myasthenia gravis or significant anemia were not recorded in our case, whose symptomatology was dominated by the pulmonary parenchymatous and cardiovascular involvements. Concerning the latter, ECG signs of pericardial and myocardial affection were present already in the autumn of 1965 in terms of inverted T waves. Superior vena cava obstruction was clinically indicated by upper venous stasis, while ankle edema and hepatomegaly were suggestive of rightsided heart failure as well. This could possibly be attributed to difficulties in diastolic filling due to pericardial and myocardial engagement, but split second sound with accentuated pulmonic component and rSR configuration in lead V 1 were consistent with an element of pulmonary hypertension too. Systolic murmur corresponding to the pulmonary artery compression has been noted in earlier cases (11) but was not heard in the present patient.

Pathoanatomical changes. The different histological appearance of the tumour tissue in the biopsy specimen (Fig. 4 *a*) and at autopsy (Fig. 4 *b*) with the complete disappearance of the lympocytic component, seems to be of considerable interest. Like the pronounced fibrotic alterations, it probably reflects effects of the radiological treatment. The lymphocytes in epithelial thymomas are considered to be immunocompetent cells (13) and not true thymocytes. Probably the host defence against the epithelial component may be influenced by the supression of the lymphocytic infiltration. However, the large areas of fibrosis and necroses in the tumour tissue at the time of autopsy may plausibly indicate effects also upon the epithelial cells of the radiological treatment.

Pulmonary hypertension. The development of cor pulmonale is well recognized in pulmonary arterial compression by aortic aneurysm (10, 21). In compression by mediastinal tumour, heart catheterization has been performed in 7 cases; pronounced central pulmonary hypertension called forth by the constriction was present in 4 (2, 9, 20). They all had a tumour other than thymoma. In the remaining 3 thymoma cases,



Fig. 5. The heart. Note tumour growing in the epicardium and infiltrating the myocardium.

moderate right ventricular hypertension was present in 1 (16) and essentially normal pulmonary arterial pressures with pressure gradient over the constriction of 10–20 mmHg in 2 (19 22) Although slow contrast passage was noted at angiocardiography in one of them, none appeared to have peripheral pulmonary arterial or venous narrowing. A discrepancy in severity of pulmonary arterial compression between thymomas and other mediastinal tumours with this complication may perhaps reflect the particular tendency of thymoma to spread to lung structures rather than in the anterior mediastinum (11 16, 18)

In our case there was moderate pulmonary hypertension. However several mechanisms may be considered.

Heart constriction. Pressure curves from the right atrium and right ventricle were consistent with right ventricular constriction, probably secondary to the perimyocardial engagement (Table I) Both in myocardial and pericardial disease a pressure increase in the lesser circulation may occur as well as typically parallel elevated PCV and pulmonary arterial diastolic pressures (6) but these were not raised in our case Even in severe constrictive pericarditis, systolic pulmonary arterial pressures seldom exceed 40 mmHg (6)

Pulmonary arterial compression Although it is now believed that there is a linear relationship between pulmonary arterial pressure and flow (7) the flow must rise about 4 times to double the pressure. Therefore increased flow through the unobstructed right pulmonary artery in the present case *offers a satisfactory explanation of the* pulmonary hypertension.

Tumour growth into the pulmonary vessels Speculatively intraluminal tumour growth might account for these changes However infiltration into the lumina of pulmonary vessels seems to be very rare in thymomas (11) and could not be traced in the present case.

Pulmonary venous compression. There were angiographic indications of left pulmonary venous obstruction and this may be a contributory factor since bilateral pulmonary venous obstruction may lead to pulmonary hypertension (4) and unilateral venous obstruction to unilateral pulmonary arteriosclerosis (12)

Unilateral hypoxia—unilateral vasoconstriction Finally even if there was no general hypoxia, impaired pulmonary function on the left side appears plausible and it is well known that local pulmonary hypoxia leads to corresponding, local pulmonary vasoconstriction (1).

In conclusion, we think that many factors may have contributed to the pulmonary hypertension in the present case.

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RECURRENT PULMONARY EMBOLISM—INCIDENCE, PREDISPOSING FACTORS AND PROGNOSIS

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Abstract. A series of 33 patients with recurrent pulmonary embolism (RPE) collected during 14 years has been compared with all 145 patients with pulmonary embolism (PE) during one year in the same hospital concerning age distribution, sex ratio and predisposing diseases or states. The symptoms and signs of RPE and the results of heart catheterization and blood gas analyses are discussed. Dyspnea and hyperpnea were predominant symptoms and the grade of dyspnea was related to the mean pulmonary artery pressure ($\bar{P}_{PA}$), hich also had strong bearing on the prognosis. PaO_2 and SaO_2 decreased ith increasing $\bar{P}_{PA}$ and with $\bar{P}_{PA}$ above 50 mmHg the chances of recovery seem small. However one case is reported with fairly good outcome, marked fall in $\bar{P}_{PA}$ and decreasing pulmonary vascular resistance during long-term dicoumarol treatment.

In two independently conducted studies on recurrent pulmonary embolism (RPE) this disease was shown to present itself as two main clinical types (17 49). Later studies have confirmed these findings. It is commonly considered that one type, characterized by gradually increasing dyspnea, primarily during exercise and later at rest, is due *to recurrent microembolism whereas the other* type, characterized by stepwise increasing dyspnea, is connected with clinically more or less evident attacks of pulmonary macroembolism.

RPE is usually considered to be an uncommon disease entity but data are lacking as regards its incidence, sex ratio and age distribution. Its connection with diseases and states considered as predisposing to thrombosis and embolism is also unclear. The present study is concerned with analysing some of these details in a series of patients with RPE. A comparison is also made with the findings from a clinical series with mainly single episodes of pulmonary embolism (PE) during one year.

As the RPE series has been collected during 14 years, some factors of prognostic importance ha e emerged. One patient, who showed a surprisingly fa ourable outcome with decreasing pulmonary vascular resistance over a period of 7 years, will be reported in this paper in greater detail.

PATIENT SERIES

Göteborg has about 360 000 inhabitants abov 15 years f age. This population is served essentially by one hospital, Sahlgren Hospital, for acute diseases. The medical and surgical services of this hospital have yearly around 26 000 admissions.

The present study comprises tw series of patients. The first (A) consists of all the 145 patients treated for PE as primary or secondary diagnosis in any of the medical or surgical wards dur ng the year 1967

The second series (B) contains the patients ith RPE who were treated in the same clinics during the years 1957–70. This series contains 33 patients. Through collaboration ith the clinicians and pathologists of the hospital we have tried to find as many as possible of the RPE cases during the years in question, but the figures should be viewed as minimum.

The A series from 1967 includes six cases f RPE, but on retrospective analyses of this series no earlier unknown cases of RPE were found.

METHODS

The A series as analysed retrospectively. The records of all patients in 1967 with clinical diagnosis of PE when postmortem examination had demonstrated PE were reviewed. The patients were classified into definite, probable and suspected cases of PE. The criteria used for this division ere typical clinical history ith sudden onset of dyspnea, hyperpnea, chest pain, hemopty sis combined with decreased arterial oxygen tension (PaO_2) or saturation (SaO_2) and low carbon dioxide tension ($PaCO_2$), together with typical ECG, heart and lung X-ray scintigram ith I^{131}-albumin, scanning ith Xenon^{133} and or pulmonary angiogram.

Table I. *Summary of the case histories in the B series (RPE)*

Case no	Sex	Born	Age at first pulm. sympt. (y.)	Duration of pulm. sympt. (y.)	Died	Episodes of periph. thrombosis	Episodes of PE	Dyspnea	Hyperpnea	Chest pain	Syncopal attacks	Other diseases
1	♂	1890	65	6	1961	(+)	+	+++	+++	++	+	
2	♂	1926	27	6	1959	+	+	+++	++	++	−	Leg fracture
3	♂	1913	43	½	1957	+	+	+++	+	(+)	−	
4	♂	1937	18	11	1966	+	+	+++	+	+	−	Prim. thrombocythemia
5	♂	1890	66	5	1961	−	+	+++	+++	(+)	−	Accident + bed rest
6	♀	1888	66	6	1962	+	+	+++	++	−	−	
7	♂	1892	68	½	1960	+	+	++	+	+	−	
8	♀	1899	60	1	1960	−	−	+++	++	−	−	
9	♂	1906	54	½	1961	−	+	++	+	(+)	−	
10	[illegible]	1919	44	1	1964	−	+	+++	++	++	−	
11	♂	1925	36	3	1964	−	−	+++	+	+	−	
12	♂	1919	43	4½	1966	+	+	+++	++	+	−	Leg fracture
13	♂	1901	57	9	1964	−	+	+++	++	+	−	
14	♀	1889	77	½	1965	+	+	+++	++	+	−	
15	♂	1910	57	½	1967	−	+	++	++	+	+	
16	♂	1896	62	6	1964	−	+	+++	+	+	−	
17	♂	1912	50	5	1967	+	+	++	++	+	−	Multiple arterial thromboses
18	♀	1941	26	1	1967	−	+	+++	++	+	−	
19	♂	1900	67	½	1968	+	+	++	++	−	+	
20	[illegible]	1933	30	6	1969	−	−	+++	+	−	+	
21	♂	1945	20	1	1966	−	−	+++	+	+	−	
22	♂	1908	58	4	1970	−	+	++	−	+	−	Mitral and aortic valv. disease
23	♀	1900	67	1½	1968	−	+	−−	−	+	−	Prim. polycyth.
4	♀	1900	66	2	1968	−	+	+++	+	+	−	
25	♂	1931	29	7		−	+	+++	+	++	−	
26	♂	1899	6[illegible]	6		−	+	+	−	−	−	
[illegible]	♂	190[illegible]	60	5		−	+	+	+	−	−	
	♂	1919	38	10		+	+	++	(+)	+	−	
[illegible]	♂	1908	60	1		−	+	+	−	+	−	
		1927	31	13		−	+	+++	−	−	+	
[illegible]	♂	1926	41	3		−	−	++	−−	+	+	
32		1915	41	14		−	+	++	+	+	−	
33		1919	43	9		−	+	+	+	+	−	

If three or more episodes of PE were diagnosed the term RPE was used.

The B series was on the whole examined much more carefully. Most of the cases were analysed when alive and some were followed for several years. Episodes of possible peripheral venous thrombosis and PE were asked for in nearly all cases. Dyspnea, hyperpnea and chest pain were graded on a 4-degree scale from − to +++. The number of syncopal attacks was also noted.

Physical findings, such as accentuated second pulmonic sound and signs suggesting right heart failure (3-grade scale) and ECG signs of right ventricular hypertrophy (3-grade scale), were recorded.

Scintigraphy with I[131]-albumen began to be used in the hospital in the mid 1960's. At about the same time it became possible to perform lung scanning with Xenon given i. and by inhalation.

Most lung X-rays were read by the same roentgenologist. They were especially analysed for the presence of parenchymal densities in the lung fields, irregular peripheral vascular markings and dilatation of the central pulmonary arteries (49). Finally the roentgenologic heart volume in ml/m² BSA was determined according to Jonsell (25). Pulmonary angiography was performed in some patients and the details have earlier been described (49).

At heart catheterization the pressures were usually recorded in the pulmonary artery (PA), left atrium (LA) or pulmonary wedge position (PCV) with simultaneous measurement of cardiac output ($\dot{Q}$) by the dye dilution technique using cardiogreen and/or bromsulphthalein as indicator. The pulmonary vascular resistance (PVR) ($(P_{PA} - P_{LA})/\dot{Q}$), or total pulmonary resistance (TPR), $P_{PA}/\dot{Q}$ have been calculated.

Table II. *Summary of clinical X-ray and postmortem examinations in the B series (RPE)*

Clinical examination						X-ray					Postmortem		
Case no	Acc. P	Right heart failure	ECG Right ventr hypertrophy	131I scanning	Xenon scanning	Parench. dens. in the lungs	Irreg. perf pulm. vessels	Dilat central pulm. arteries	Heart size (ml/m² BSA)	Pulm. angio.	Large pulm emb.	Small pulm. emb	Peripheral thromboses
1	+	++	+			+	+	–	600		+	–	+
2	+	+	++			+	+	+	640		+	+	–
3	+	(+)	++			–	+	+	720		–	–	+
4	+	+	+			+	+	+	750	+	–	–	
5	+	++	+			+	+	+	440		–	–	–
6	+	+	+			–	+	+	enlarged		–		
7	–	–	+			+	+	–	440				
8	–	–	–								+	–	–
9	–	+	–			+	+	+	430		–	+	
10	+	++	+			–	+	+	520		–	+	–
11	+	++	+			–	–	+	470	+	–	+	–
12	+	++	+			+	+	+	600		–	+	–
13	–	++	–			+	+	+	540	–	–	–	+
14	+	++	–								–	–	–
15			+								+	–	–
16	–	++	+			+	+	+	500		–	+	–
17	+	+	+			+	+	+	360		–	+	–
18	+	+	+			+	–	+	500		–	–	–
19	+	++	+			–	–	+	680		+	+	+
20	+	++	++	+	(+)	–	+	+	570				
21	+	+	++			–	–	+	570		–	+	–
22	+	+	–	–		+	–	+	790		+	+	–
23	+	+	–	+	+	+	–	–	460		–	+	–
24	+	–	+	+	+	+	+	+	900	+	+	+	+
25	+	–	+			+	+	+	330				
26	–	–	–			–	+	+	480				
27	–	–	+			+	+	+	470				
28	+	( )	+			+	–	+	630				
29	–	–	–	+		+	–	–				Op. +	
30	+	–	++	–		–	–	+	380	+			
31	+	–	+	+	+	+	–	–	390				
32	–	–	–			+	–	–	450				
33	–	–	+	+	+	–	–	–	410				

Arterial blood gases during breathing of room air were usually determined at the heart catheterization but sometimes the samples were also taken on other occasions.

The certainty of the diagnoses differs for the different patients in this series (Tables I–II). Twenty-four of the 33 patients in series B have died. Multiple pulmonary emboli were found in all the 22 autopsy cases. In the two cases, in which autopsy was refused, the diagnoses were certain from the typical clinical picture. Nine patients are alive and have been diagnosed as RPE after thorough investigations and much discussion. In cases when heart catheterization or pulmonary angiography were not performed a series of typical embolic episodes have been required for a positive diagnosis.

Malignant tumours, recent surgical operation, trauma, heart disease (especially heart decompensation), cerebral disease with paresis and certain blood diseases have been regarded as predisposing conditions for PE. Their occurrence in the two series has been analysed.

RESULTS

One year incidence of pulmonary embolism

Most PE cases, particularly in the higher age groups, were diagnosed at the postmortem examination (Fig. 1). The sex distribution was equal for the different age groups with the exception of the ages 40–49 for which there was an excess of men (Fig. 2). Among the latter there also seems to be a lack of predisposing factors. Five cases of RPE were recorded. Only one patient, an 18-year-old woman used contraceptive pills and had a suspected PE after appendectomy. Above 50 years of age PE was connected with operation and trauma in 27–40% of the cases (Fig. 3). In higher age groups there were more cases of PE and a comparatively larger num-

Table III. *Results of heart catheterizations and blood gas analyses in the B series RPE (cases 1–24 have died)*

Case no	Examined in (yr)	R/E	Heart catheterization $\bar{P}_P$ (mmHg)	$\bar{P}_{LA}$ (PCV) (mmHg)	Q (l/min)	PVR (TPR)	$PaCO_2$	SaO_2	PaO_2
1	1961	R	59	—	4.3	(13.7)	34	89	53
2	1959	R	—	—	—	—	26	88	(55)
		200	—	—	—	—	24	91	(61)
3	1957	R	61	(4)	4.3	14.2	—	81	(46)
		E	78	(6)	—	—	—	78	(43)
4	1961	R	32	—	—	—	—	—	—
5	1956	R	—	—	—	—	40	86	59
6	1961	R	—	—	—	—	31	87	(55)
7	1960	R	—	—	—	—	27	88	(54)
8	1960	—	—	—	—	—	—	—	—
9	1960	—	—	—	—	—	—	—	—
10	1963	R	50	2	4.2	11.5	33	94	75
		100	58	5	5.9	9.0	26	90	67
11	1963	R	49	0	3.8	12.9	25	98	73
		100	67	2	4.7	13.9	24	88	50
12	1964	R	50	2	3.8	12.7	34	95	71
		100	65	1	5.3	12.1	31	96	78
13	1961	R					29	82	
14	1966	—	—	—	—	—	—	—	—
15	1967	—	—	—	—	—	21	83	
16	1963	R	70	—	3.7	(18.9)	36	80	—
17	1963	R	29	—	3.4	(5.4)	31	94	(71)
18	1966	R	44	2	2.2	20.0	28	92	56
19	1968						34	96	
20	1969	R	60	5	2.1	26.2	27	86	
21	1966	R	83	4	3.6	21.9	33	91	59
22	1970	—	—	—	—	—	—	—	—
23	1968	R	—	—	—	—	31	96	80
24	1968	R	46	—	2.7	(17.0)	24	93	
25	1961	R	17	(8)	5.7	3.0	—	97	(90)
		E	15		9.2	(1.6)	—	96	(85)
	1963	R	16	(5)	6.5	1.7	35	95	83
		200	29	(9)	11.9	1.7	36	96	91
27	1963	R	23	(5)	5.4	3.7	32	93	(68)
		200	34	(6)	5.9	[illegible]	34	94	(74)
28	1962	R	26	6	9.4	2.1	33	97	67
		300	44	6	14.0	2.4	32	90	55
29	1968								
30	1962	R	59	(8)	6.4	8.0	28	93	72
	1969	R	34	—	7.6	(4.4)	31	99	82
31	1967	R	9	7	6.6	0.3	35	98	103
		600	18	—	—	—	30	100	115
32	1967	R	—	—	—	—	33	99	106
33	1968	R	—	—	—	—	30	100	85

R = rest. E = exercise (kpm/min).
Values calculated from the Dill curve given within parentheses.

ber were combined with malignant tumours. Above 60 years of age 24 out of 37 operated patients who developed PE were operated on because of malignancy. Below 50 years of age on the contrary none of the operations were connected with malignant disease. Malignancy, operation or trauma were present in 50% (7[illegible] of 145 cases). When cases with cardiac or cer[illegible] diseases were added, 81% of the patients had one or more of the diseases considered as predisposing to PE. Above 80 years 19 of 53 patients with PE had cardiac or cerebral diseases and in the ages 70–79 years such diseases were found in 23 of 46 cases. PE seemed to occur a fairly short

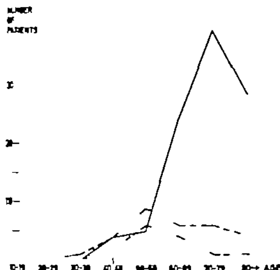


Fig. 1 Ways of diagnosing pulmonary embolism in the A series (1-year series of PE). Diagnosis at autopsy (——), clinically confirmed (– –), clinically probable or suspected (–).

time after the cerebral accident—also after subdural hematoma. Thus it was not related to a long bed rest.

Eighteen of the 27 patients who did not have any of the predisposing diseases listed in Fig. 3 had other diseases, such as myelofibrosis (1 case), hemolytic anemia (1 case) infectious diseases (3 cases), glomerulonephritis (1 case) and chronic alcoholism with fatty liver (3 cases) etc. Thus, in only nine of these patients was PE found as single disease.

Out of the 145 patients in this series 105 died.

Recurrent pulmonary embolism

The age distribution of the 33 cases with RPE is compared with the age distribution of the series of patients with PE in 1967 To mak the age comparison as correct as possible, the age at the first hospital examination was used for the cases with RPE. As seen from Fig. 4 RPE has a more even age distribution than PE. Most cases were between 40 and 70 years old. Up to 70 years of age there was a predominance of men over women.

Other diseases or states which might be predisposing to venous thrombosis and PE were found in a few cases (Table I). In three cases fractures or accidents could possibly have precipitated the embolism. A severe primary throm-

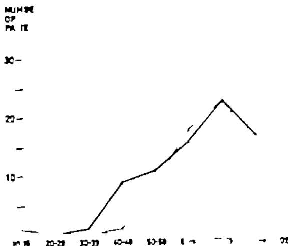


Fig. 2. Age and sex dist butio m [illegible] ——— men; ——— omen.

bocythemia was probabl cf [illegible] s gnificance in one young man a [illegible] primary polycythemia in anoth [illegible] ged man suffered from a series of arte [illegible] d [illegible]ous thromboses during several years A [illegible]s of hospital examinations was performed but [illegible] explana tion was found. One man had mitral [illegible] aortic val ular disease

Symptoms. Nineteen of the 33 patient had had clinically diagnosed thromboses o signs suggest ing thrombosis. In most instances these thrombotic episodes were however not connected with the occurrence of signs suggesting pulmonary em-

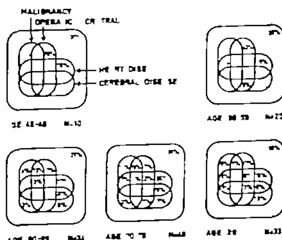


Fig. 3 Venn diagrams giving the occurrence of factors regarded as predisposing for PE in different age groups of the A series.

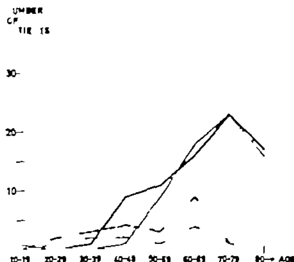


Fig 4 Age and sex distribution in the A series (1-year series of PE) compared with the B series (RPE). ——, A series, men; —— A series, omen; --- B series, men, ---, B series, women.

bolism. Thirty of the patients had had symptoms which retrospectively suggested PE. These symptoms had usually not been so severe that the patients had sought medical advice or that the physician had suspected PE. With progress of the disease dyspnea and hyperpnea were the most characteristic symptoms (Table I) Seventeen of e 24 patients who died had very severe dyspnea de + + +) Of the nine who are alive, only had dyspnea of that severity A similar difference for hyperpnea was also found.

Chest pain as not a dominating complaint. Only four atients had pain of moderate degree and anothe wo with severe pulmonary hyper tension com d of chest oppression. In some of the cases ain could hardly be differentiated from angi pe toris

Syncopal atta we e registered in six cases and only on sing ns. They were usually precipitated by no but not necessarily of severe degree. He pt occurred in only two cases.

In the 24 cases wh died the duration of symptoms varied between 6 months and 11 years (mean 3.4 years). The age at death varied from 21 to 77 years (mean 54) Those who are alive have been followed between 1 and 14 years (mean 7 6). They are now between 40 and 72 years of age (mean 55).

Signs and laboratory findings An accentuated second pulmonic sound was registered in 22 cases and signs of right heart failure in 14 (10 with grade + + and 11 with grade + Table II)

Marked signs of right ventricular hypertrophy in the ECG (+ +) were present in five cases—four of them died later—whereas 19 cases had right ventricular hypertrophy of moderate degree (+). The latter finding was as common among those who died as among those who still are alive.

Scanning with J^{131}-albumen or Xenon was performed in eight cases and was positive in six of them. The two methods showed close conformity in all cases. In one patient in whom the Xenon test was positive the J^{131} test did not demonstrate any region with decreased perfusion.

Parenchymal densities of unspecific kind in the lung fields on plain X rays were found at one time or another in 21 of the 33 cases. The X-rays were quite normal in 10 cases and could not be elucidated in the remainder Irregular vascular markings were present in 18 cases on plain X rays, most of these changes were found in the patients who later died. Dilatation of the central pulmonary arteries was present in 18 of the 24 cases who died and in five of the nine who are alive. The data do not permit any conclusion as to correlation between this finding and the pulmonary artery pressure.

The highest limit for normal heart volume is 450 ml/m² BSA. The heart was enlarged on X ray examination in 16 of the 24 cases who died and in three of the nine who are alive. The mean values were 554 ± 116 ml/m² BSA and 445 ± 88 ml/m² BSA ($p < 0.025$).

Table IV *Data obtained at heart catheterization among cases who died and cases who are alive in the B series (RPE)*

The numbers differ because all investigations were not performed in all cases
— no. of cases, $\bar{x}$ — mean value, — standard deviation

	Patients who died			Patients who are alive			
		$\bar{x}$			$\bar{x}$		$p<$
Pr	12	52.9	15.0	6	25.3	17.6	0.005
PVR or TPR	11	15.9	5.7	6	3.1	2.7	0.001
SaO_2	19	89.4	5.5	8	96.5	2.6	0.001
PaO_2	13	62.1	10.5	8	84.3	15.0	0.001
$PaCO_2$	17	29.6	5.1	7	32.3	2.6	0.05

Pulmonary angiography was performed in eight cases and showed obstructive changes in all of them.

Heart catheterization was performed in 18 cases at rest on at least one occasion and in nine cases also during supine exercise (bicycle ergometer). The means for some data at heart catheterization (pressures, PVR and blood gases) are given in Table IV.

In the patients who later died P_{PA} was significantly higher, SaO_2, PaO_2 and $PaCO_2$ significantly lower than in the patients who are alive. The patients with dyspnea of grade + + + had significantly higher P_{PA} and lower PaO_2 and SaO_2 than those with lower grade of dyspnea. There was no significant difference with respect to $PaCO_2$.

The $\bar{P}_{LA}$ or pulmonary capillary venous pressures (PCV) were normal in all cases in which they were determined. As mentioned above, signs of rheumatic heart disease were present only in one case.

There was a significant correlation between $\bar{P}_{PA}$ and SaO_2 ($r = 0.65$, $p < 0.005$) and between PVR and SaO_2 ($r = 0.65$, $p < 0.005$), and similar correlations between PaO_2 and P_{PA} (Fig. 5) and PaO_2 and PVR (Fig. 6), $r = 0.73$ and 0.71 respectively. There was no significant correlation between $\bar{P}_{PA}$ and $PaCO_2$ and an almost significant correlation between PVR and $PaCO_2$ ($r = 0.49$, $0.10 > p > 0.05$). PaO_2 and $PaCO_2$ were not significantly correlated to each other.

The reaction to physical exercise in cases 3, 10, 11, 12, 25, 26, 27, 28 and 31 is seen from

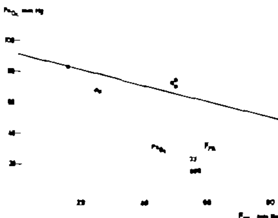


Fig. 5. Relationship between $\bar{P}_{PA}$ and PaO_2 in 14 cases with RPE.

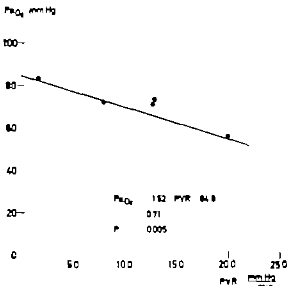


Fig. 6. Relationship between PVR and PaO_2 in 14 cases with RPE.

Table III. In all but cases 25 and 31 the $\bar{P}_{PA}$ was higher than normally accepted during exercise. The PVR remained high during exercise in those cases who later died, but fell in some of those who are alive. There was a tendency to decreasing SaO_2, PaO_2 and $PaCO_2$ during exercise among those who had high P_{PA}.

In some cases (nos. 11, 12, 27 and 30) heart catheterization was performed on two occasions with some years' interval. In patients who later died there was a steady progress of the pressures. Patient 27 had fairly low pressures on two occasions. Patient 30 showed an interesting development, and her history is given below.

CASE REPORT

Case 30. A married woman without deliveries. In 1958, when she was 31 years old, she suffered from severe breathlessness and fainted during a bicycle ride. Before that she had not experienced any symptoms, but after this attack she developed increasingly severe symptoms. Examinations in 1962 revealed pulmonary hypertension ($\bar{P}_{PA}$ 59 mmHg and PVR 8.0 U).

At that time she was put on long-term dicoumarol treatment. The condition improved considerably after 3–4 years' treatment. From being in functional class III (N.Y.H.A.) she is now in class I, working full time as hospital orderly and can walk and take bicycle rides without any complaints. At a new heart catheterization in 1969 P_{PA} was 34 mmHg and PVR 4.4 U. She has never had any signs compatible with peripheral thrombosis.

ase ... be cor ... d ... lth, for example, patient ... had ymptoms after ... leg a similar degree ... dyspnea and P ... 50 mmHg VR 1 9 U H ... as treated with dicoumarol as ... as the ... ther patient, but nevertheless deterio- d died

DISCUSSION

... objective was not to study the total number ... nd ... urse of event ... of PE in the population but ... c llect a series of patients with non-recurrent E f r comparison with the series of patients ith RPE.

The clinical recognit on of PE is difficult. In e eral series of pa ients found at postmortem to have PE, the diagnosis has been made ante mortem in less than 50% (6 18 24 36). This was also confirmed in our study f the 1 year patient series in which 100 of 145 PE cases were not diagnosed until the postmortem xamination (Fig 1). Most of them were elderly patients who had other diseases with symptoms concealing those of the embolism. Thus, even retrospectively it seemed difficult to make his diagnosis during life on clinical data alone.

The overall sex ratio a equal which was also found by Borgstr m (3) Hume (23 and Fejfar et al. (15). Belo ... age of 60 however we found an overrepresentation of males—21 males against 11 femal ... Fig.) Because only two ... men below 50 ... ears of age were found to ... e PE ... also analysed the records from the two Departments of Gynecology and Obstetrics during the ... me year. Only one case below the age of 50 was found to have had PE (after legal abortion). Thu ... no other case of PE in connec tion with pregnancy was registered in 1967 among 5 925 deliveries.

There is experimental and clinical evidence of connections between oper tion and trauma, on the one hand, and PE ... n the other and especially between malignancy and PE (1 ... 8 16 22, 27). A relati ely high frequency of ... enous thrombosis and PE in adults with heart diseases is also well established (26 30 44). Thus the ... enous congestion in cardiac decompensation has been thought to be of importance as well as reduced arterial perfusion in lower limbs and increase of venous blood volume in cases with low cardiac output. Some reports claim a hypercoagulable state in patients with ischemic heart disease and during treatment of heart failure with diuret... (13 ... 3 51).

An increased incidence of thromboemboli... following cerebral injuries has been reported ... Chalka et al. (7).

Most of our patients in the A series were o... 60 years of age. Among elderly patients there ... an accumulation of predisposing factors and i... difficult to evaluate which is the most importa... Bed rest and age taken together are shown to ... a great risk (37).

The present findings of an even age distri... tion in cases with RPE are validated by a co... parison with the series reported by Goodwin et ... (17) and Widimsky et al. (45), in which 74 ... 70% respectively were below 50 years of age compared to 42% in the present series. The p... centage of women was 74 55 and 33, resp... tively in the three series. However these se... of patients may have been collected from dif... ent types of hospital populations, for which r... son the differing sex ratios are difficult to co... pare. Yet they differ from the high female/m... ratio (4 1) seen in primary pulmonary hyp... tension (41).

There is a striking difference between the ca... of RPE and those with single embolism regard... predisposing diseases or states. This differe... cannot be explained by the lower age of th... with RPE. Our results cannot be explained b... bias in looking for these predisposing disea... because these states were looked for more ca... fully in RPE than was possible in the retros... th e 1 year series of PE.

In 19 of 33 cases with RPE signs sugges... leg thrombosis were found on some occasion. ... earlier described by Widimsky et al. (45), s... of disturbed leg vein function are a common f... ing in RPE. In earlier studies of RPE, pre... posing diseases or states such as axillary ... thrombosis (14) or ingestion of contracep... drugs are described (32).

Pandolfi et al. (35) have shown that pati... with vein thrombosis have lower fibrinolytic ... tivity in the walls of veins. Disturbances of ... type may be one cause of the increased tend... to embolism in the cases with RPE. Detailed s... ies of blood coagulation and fibrinolysis w... however only performed in a few of our c... with RPE and in none were studies made of fibrinolytic activity of the walls of veins.

As pointed out both by Goodwin et al. (17) by Wilhelmsen et al. (49), two clinical types of RPE may be differentiated. In one group of [illegible] clinical episodes of PE can often be [illegible]ed and autopsy often shows emboli of the [illegible] pulmonary arteries. In the other group [illegible]ical episodes suggesting PE are lacking and most cases postmortem examination indicates [illegible] of smaller vessels. Gradually increasing [illegible]pnea is the most typical symptom in this group. [illegible]erwise no discriminating or predisposing fac[illegible] was found that might explain the differences [illegible]ween the groups.

Dyspnea and hyperpnea are often prominent [illegible]ptoms in RPE. The results of this study indi[illegible]e that the grade of dyspnea is related to the [illegible] at rest. It has also some prognostic signifi[illegible]. Indeed the dyspnea of RPE is one of the [illegible]st severe forms of dyspnea seen clinically [illegible] decrease of PaO_2 is not of such degree that dyspnea can be explained by the anoxic drive. [illegible] left heart disease with increased $\bar{P}_{PA}$ a simi[illegible] form of dyspnea does not appear until the [illegible] is much lower. There may be some dis[illegible] of lung mechanics—increased airway [illegible]stance but normal compliance (48)—but not such a degree as to explain the dyspnea. A [illegible]ible explanation might be a direct effect of emboli on some receptors of the vascular tree on the interstitial tissue of the lungs. No vas[illegible] receptors have been clearly demonstrated, the demonstration of type J receptors in the [illegible]rstitial tissue is interesting as it reveals a pos[illegible] afferent end-organ for this sensation which [illegible] linked to hyperpnea and can be abolished by [illegible]al blocking (34).

Chest pain syncopal attacks and hemoptysis [illegible] common complaints in acute PE but rare in [illegible]PE. Goodwin et al. (17) reported a fairly high [illegible]quency of chest pain and hemoptysis in their [illegible]oup of patients with large emboli. These symp[illegible]ms were, however not prominent in our series. The mechanism of pain has been discussed [illegible]her (18, 49).

[illegible]yncopal attacks have been found to be very [illegible] in primary pulmonary hypertension (4) [illegible] have also been encountered in some of our [illegible] with RPE. In one case asystole suddenly [illegible]eared during supine exercise (100 kpm/min) [illegible] heart catheterization. The patient quickly re[illegible]ered without any sequelae. There is no certain explanation of these attacks or of the apparent difference between RPE and primary pulmonary hypertension in this respect. They do not seem to be directly related to the $\bar{P}_{PA}$ or any other hemodynamic [illegible]ariable. Dressler (12) suggested a reflex mechanism with neuroreceptors in the wall of the pulmonary artery using the vagus nerve as an afferent pathway and resulting in a fall of the systemic blood pressure and inhibition of the sinus node. Pulmo-coronary reflexes as cause of acute cardiac standstill have been discussed by Gorham (18). According to Vatner and van Citters (40) the coronary blood flow does not fall after *acute* pulmonary embolism in experimental animals.

The present findings concerning respiratory function in RPE are in good agreement with earlier findings extensively discussed by Wilhelmsen et al. (48). PaO_2 and SaO decrease with increasing dyspnea and $\bar{P}_{PA}$ but $PaCO_2$, even though lower than normal, does not correlate sig[illegible]nificantly with $\bar{P}_P$. Suspicions as to RPE are strengthened by the findings of low PaO_2 and $PaCO_2$.

The low PaO_2 can be explained by anatomical shunts, disturbed ventilation/perfusion ratio and possibly to some extent by diffusion abnormalities (19 38 48). Orell and Hultgren (33) ha[illegible]e demonstrated shunts in autopsy preparations from patients dying from PE.

As earlier stated in this paper we have found decreased lung conductance in RPE without in[illegible]dication of functional airway constriction (48). Emphysematous changes in embolized lungs from patients apparently free from bronchitis have been observed (5 48). A connection between RPE and emphysema is, however not proved by these studies. As $PaCO_2$ generally is low in RPE, emphysema could not be a major determinant of the blood gas disturbances.

The decreased PaO in RPE will stimulate breathing and may at least partly explain the decreased $PaCO_2$. As earlier pointed out, breathing may be stimulated by the direct effect of the emboli on lung structures via, e.g. the J fibres. Stein et al. (38) have demonstrated different effects on gas exchange from micro- and macroemboli in acute experimental PE. It is probable that similar differences are present in RPE.

Diagnostic methods in PE have been discussed in several earlier papers (10 17 21 39 42, 45

49). ECG and plain X-rays are commonly considered to be too unspecific, whereas lung scintigraphy has become popular. The presence of other lung diseases—especially emphysema—may invalidate the method. In these cases Xenon scanning after both inhalation and injection is helpful. In RPE with small emboli scattered over nearly the whole lung fields even this method may fail. Lung angiography can disclose these occlusions especially if selective X-rays after injections of contrast medium into different lobal arteries are made.

In patients with a history, clinical signs and blood gases suggesting RPE, heart catheterization and pulmonary angiography should be performed to establish diagnosis and aid prognosis.

The pulmonary hypertension in RPE is considered to be solely due to mechanical factors (46, 48), but it has not been possible to correlate the number of clinical emboli to P_{PA} (45). In the present study it was found that P_{PA} had a strong bearing on the prognosis. With a P_{PA} above 50 mmHg there seems to be very small chance of remission, which may occur when P_{PA} at detection is lower (11, 47, 49, 50). The development of the case history reported in the present paper, in which P_{PA} fell from 59 to 34 mmHg with corresponding decrease in PVR, is very interesting. Other patients have been treated with dicoumarol as carefully as this patient, but he suffered progressive disease and died. Venous thromboses or PE have not been seen in any of these cases. We do not know which factors are most important for the possibility to recanalize vessels or form collaterals, and thereby for the prognosis in cases when new emboli are prevented. The possibility to stop new embolization by giving dicoumarol has been questioned.

Treatment with urokinase or streptokinase has given encouraging results in acute PE, but not in RPE, in which most of the emboli are old and cannot be influenced by these drugs (29).

Ligation of the vena cava has been recommended (9, 31, 43), but even this method has failed to prevent recurrencies (20).

Thus there is still a series of important problems to solve concerning treatment and, most important, concerning prevention of RPE. Improved methods for analysis of the increased tendency to formation of thrombosis seem most urgent. The whole question of the function of the blood coagulation and the fibrinolytic system has to be analysed in greater detail in connection with both PE and RPE.

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